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Exhibit A

CURRICULUM VITAE

Scott Southwood

Date of Birth: October 25, 1962

Place of Birth: Canton, OH

Nationality: U. S. Citizen

Address: 10679 Strathmore Drive
Santee, CA 92071
(619) 258-9549

Education:

1983 - 1985 San Diego State University
Department of Biology
B. S. (Biology)

1980 - 1982 University of Akron
Department of Biology
Akron, OH

Relevant Research Experience:

1989 - present Associate Scientist; Epimmune, Incorporated
San Diego, CA

Studies involve development of molecular binding assays for purified HLA class I and II MHC molecules. Research includes application of binding assays to characterizing specificity between MHC-peptide interactions. Experienced in size exclusion and reverse-phase HPLC. Current work has been focused on conversion of existing binding assays to a high throughput screening (HTS) platform.

Related work includes computer scanning of protein sequences for allele-specific, motif-bearing peptides. Additionally, work on the development of predictive algorithms (PIC) has been used to identify peptides with a given binding affinity potential.

Exhibit A (Southwood)



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| Author(s): | <u>Scott Southwood</u> | <u>Scott Southwood</u> | <u>10/3/98</u> |
| | <u>Elissa Keogh</u> | <u>Elissa Keogh</u> | <u>10/7/98</u> |
| Technical Review By: | <u>John Fikes</u> | <u>John Fikes</u> | <u>10/7/98</u> |

TITLE: Identification of HLA-A2 restricted epitopes for CEA, p53, MAGE2/3 and Her2/neu.

AUTHOR(S): Scott Southwood and Elissa Keogh

ABSTRACT:

HLA-A2 supertype degenerate peptides were identified from naturally occurring protein sequences of the tumor-associated antigens CEA, p53, Her2/neu, and MAGE2/3, utilizing the A2 supertype main anchor motif (LIMVAT_{2,c}) in conjunction with a customized computer program. Motif-positive peptides were synthesized and tested for binding capacity to purified A*0201, and high binders were then tested for binding to A*0202, A*0203, A*0206, and A*6802 MHC molecules. A total of 46 high binding and degenerate peptides were identified: ten CEA, ten p53, seventeen MAGE 2/3 and nine Her2/neu.

Exhibit B / Southwood Declaration

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INTRODUCTION:

The goal for this screening program was to identify at least 2 epitopes for the TAAs (tumor-associated antigens) MAGE2/3, Her2/neu, CEA (carcinoembryonic antigen) and p53 which bind at least three of the five most predominant HLA-A2 supertype alleles, and are able to induce CTLs that are peptide-specific, and recognize tumor targets endogenously expressing the antigen.

CEA is a 180kD cell surface and secreted glycoprotein produced by a number of different tumors, particularly colon cancer, but in a normal setting is associated with fetal tissue (Cancer Biology, 3rd ed., R. Ruddon, 1995:p 126). The abnormally high expression on cancer cells makes CEA an important target for immunotherapy.

MAGE, melanoma antigen genes, are a family of related proteins whose expression is normally limited to testis and placenta but is also expressed by melanomas and a variety of other carcinomas. These proteins are known to be recognized by cytotoxic T cells (Cellular and Molecular Immunology, 3rd ed., A. Abbas, A. Lichtman and J. Pober, 1997: p. 390).

Her2/neu or erbB-2 is a 185kD transmembrane protein that is similar to EGF receptor. The Her2/neu oncogene transforms cells when it is overexpressed and has tyrosine kinase activity making it capable of auto-phosphorylation. It is expressed primarily in breast, ovarian and gastric cancers (Cancer Biology, 3rd ed., R. Ruddon, 1995: p. 302).

The fourth TAA targeted, p53, is normally a tumor suppressor gene but when mutated, becomes tumorigenic and increases cell proliferation. The most common mutations are at positions 175, 248, 273 and 282 and these mutations have been observed in colon, lung, prostate and osteosarcomas as well as other tumors (Cancer Biology, 3rd ed., R. Ruddon, 1995: p. 323). These mutations result in increased expression of both the

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wildtype and mutant p53 proteins, thereby qualifying this protein as a TAA.

Development of a multi-epitope vaccine should allow us to target colon, breast and lung carcinomas expressing these TAAs as well as having broader applications for the treatment of other carcinomas.

MATERIALS AND METHODS:

MHC sources.

The following EBV transformed cell lines were used as sources of class I molecules: JY (A*0201), FUN (A*0203), CLA (A*0206), and AMAI (A*6802). The mouse B cell lymphoma p815 transfected with A*0202 was also utilized.

Cells were maintained in vitro by culture in RPMI 1640 medium supplemented with 2mM L-glutamine and 10% heat-inactivated FCS. Cells were also supplemented with 100µg/ml of streptomycin [Irvine Scientific, Santa Ana, CA] and 100U/ml of penicillin [GIBCO, Grand Island, NY]. Large quantities of cells were grown in roller bottles.

Affinity purification of HLA-A molecules.

Cells were lysed at a concentration of 10^8 cells/ml in PBS containing 1% NP-40 and 1mM PMSF (phenylmethylsulfonyl fluoride, Sigma P-7626, St. Louis, MO). The lysates were cleared of debris and nuclei by centrifugation at $10,000 \times g$ for 20min.

MHC molecules were then purified by affinity chromatography as previously described (A. Sette et al, J Immunol 142:35, 1989). Columns of inactivated Sepharose CL4B and Protein A Sepharose were used as pre-columns. Lysates were filtered through 0.8 and $0.4\mu\text{M}$ filters and then depleted of HLA-B and HLA-C molecules by repeated passage over Protein A Sepharose beads conjugated with the anti-HLA(B,C) antibody B1.23.2 (N. Rebai et al, Tissue Antigens 22:107, 1983). Typically 2 to 4 passages were required for

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effective depletion. Subsequently, the anti-HLA(A,B,C) antibody W6/32 (C. J. Barnstable et al, Cell 14:9, 1978) was used to capture HLA-A molecules.

Independently, both antibody columns were washed with 15-column volumes of 10mM TRIS in 1.0% NP-40, PBS and 2-column volumes of PBS containing 0.4% n-octylglucoside. Finally, the class I molecules were eluted with 50mM diethylamine in 0.15M NaCl containing 0.4% n-octylglucoside, pH 11.5. A 1/25 volume of 2.0M Tris, pH 6.8, was added to the eluate to reduce the pH to ~8.0, and then concentrated by centrifugation in Centriprep 30 concentrators (Amicon, Beverly, MA) at 2000rpm. Protein purity, concentration, and effectiveness of depletion steps were monitored by SDS-PAGE.

A2 supertype molecular binding assays.

Purified human class I molecules [5 to 500nM] were incubated with 1-10nM ¹²⁵I-radiolabeled probe peptide, iodinated by the Chloramine T method (S. Buus et al, Science 235:1335, 1987), for 48h at room temperature in the presence of 1 μM human β₂ microglobulin (Scripps Laboratories, San Diego, CA) and a cocktail of protease inhibitors. The final concentrations of protease inhibitors were: 1mM PMSF, 1.3nM 1.10 phenanthroline, 73μM pepstatin A, 8mM EDTA, and 200μM N alpha-tosyl-lysine chloromethyl ketone (TLCK).

Class I peptide complexes were separated from free peptide by gel filtration on TSK200 columns; and the fraction of bound peptide calculated as previously described (A. Sette et al, J Immunol 142:35, 1989). In preliminary experiments, the HLA class I prep was titrated in the presence of fixed amounts of radiolabeled peptides to determine the concentration of class I molecules necessary to bind 10-20% of the total radioactivity. All subsequent inhibition and direct binding assays were then performed using these class I concentrations. In the inhibition assays, peptide inhibitors were typically tested at

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concentrations ranging from 120 μ g/ml to 1.2ng/ml. The data were then plotted and the dose yielding 50% inhibition was measured. Peptides were tested in two to four completely independent experiments. Since under these conditions [label]<[MHC] and IC50 \geq [MHC], the measured IC50s are reasonable approximations of the true k_D values.

Radiolabeled probe and standard control peptides used are as follows:

A F₆→Y analog of the HBV core 18-27 peptide (sequence FLPSDYFPSV; J. Ruppert et al, Cell 74:929, 1993) was used as the radiolabeled probe for the A*0201, A*0202, A*0203, and A*0206 assays. A C₄→A analog of the HBV pol 646-654 peptide (sequence FTQAGYPAL) was used as the radiolabeled probe for the A*6802 assay. The average IC50's of F₆→Y analog of the HBV core 18-27 peptide for the A*0201, A*0202, A*0203, and A*0206 assays were 5.0nM, 4.3nM, 10nM, and 3.7nM, respectively. The average IC50 of the C₄→A analog of the HBV pol 646-654 peptide in the A*6802 assay was 8.0nM.

Peptide Synthesis.

Peptides were either synthesized at Cytel Corporation (San Diego, CA), as previously described (J. Ruppert et al, Cell 74:929, 1993), or, for large epitope libraries, purchased as crude material from Chiron Technologies (Chiron Corp., Clayton, Victoria, Australia). Peptides synthesized at Cytel were purified to >95% homogeneity by reverse-phase HPLC. The purity of these synthetic peptides was determined using an analytical reverse-phase column and their composition confirmed by amino acid analysis and/or mass spectrometry analysis.

Target Cell Lines for Cellular Screening. The .221A2.1 cell line, produced by transferring the HLA-A2.1 gene into the HLA-A, -B, -C null mutant human B-lymphoblastoid cell line .221, was used as the peptide-loaded target to measure activity of HLA-A2.1 restricted CTL. The HLA-typed melanoma cell lines (624mel and 888mel)

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were a generous gift from Y. Kawakami and S. Rosenberg, National Cancer Institute, Bethesda, MD. The colon adenocarcinoma cell lines SW403 and HT-29, the osteosarcoma line Saos-2 and the breast tumor line BT549 were obtained from the American Type Culture Collection (Rockville, MD). The gastric cancer line , KATO III, was obtained from the Japanese Cancer Research Resources Bank. The Saos-2/175 (Saos-2 transfected with the p53 gene containing a mutation at position 175) was obtained from Dr. Levine, Princeton University, Princeton, NJ. All cell lines, except those from the ATCC, were grown in RPMI-1640 medium supplemented with antibiotics, sodium pyruvate, nonessential amino acids and 10% (v/v) heat inactivated FCS. The melanoma, colon and gastric cancer cells were treated with 100U/ml IFN γ (Genzyme) for 48 hours at 37°C before using them as targets in the ⁵¹Cr release and in situ IFN γ assays. The p53 tumor targets were treated with 20ng/ml IFN γ and 3ng/ml TNF α for 24 hours prior to assay (M. Theobald, et al PNAS 92:11993, 1995).

Primary CTL Induction Cultures.

Generation of Dendritic Cells (DC): PBMCs were thawed in RPMI with 30 μ g/ml DNase, washed twice and resuspended in complete medium (RPMI-1640 plus 5 % AB human serum, non-essential amino acids, sodium pyruvate, L-glutamine and penicillin/streptomycin). The monocytes were purified by plating 10 X 10⁶ PBMC in 3 ml of complete medium in each well of a 6-well plate. After 2 hrs at 37°C, the non-adherent cells were removed by gently shaking the plates and aspirating the supernatants. The wells were washed a total of three times with 3 ml RPMI to remove most of the non-adherent and loosely adherent cells. Three 3 ml of complete medium containing 50 ng/ml of GM-CSF and 1,000U/ml of IL-4 were then added to each well and the DC were ready to be used for CTL induction cultures after 7 days.

Induction of CTL with DC and Peptide:

CD8⁺ T-cells were isolated by positive selection with Dynal immunomagnetic beads and

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detachabead reagent according to the manufacturer's instructions. Typically $200\text{--}250 \times 10^6$ PBMC were processed to obtain 24×10^6 CD8+ T cells (enough for a 48-well plate). Briefly, the PBMCs were thawed in RPMI with $30\mu\text{g/ml}$ DNase, washed once with PBS containing 1% human AB serum and resuspended in PBS/1% AB serum at a concentration of 20×10^6 cells/ml. The magnetic beads were washed 3 times with PBS/AB serum, added to the cells ($140\mu\text{l}$ beads/ 20×10^6 cells) and incubated for 1 hour at 4°C with continuous mixing. The beads and cells were washed 4x with PBS/AB serum to remove the nonadherent cells and resuspended at 100×10^6 cells/ml (based on the original cell number) in PBS/AB serum containing $100\mu\text{l/ml}$ detacha-bead reagent and $30\mu\text{g/ml}$ DNase. The mixture was incubated for 1 hour at room temperature with continuous mixing. The beads were washed again with PBS/AB/DNase to collect the CD8+ T-cells.

The DC were collected and centrifuged at 1300 rpm for 5-7 minutes, washed once with PBS with 1% BSA, counted and pulsed with $40\mu\text{g/ml}$ of peptide at a cell concentration of $1\text{--}2 \times 10^6$ /ml in the presence of $3\mu\text{g/ml}$ β_2 -microglobulin for 4 hours at 20°C . The DC were then irradiated (4,200 rads), washed 1 time with medium and counted again.

Setting up induction cultures: 0.25 ml cytokine-generated DC ($@1 \times 10^5$ cells/ml) were co-cultured with 0.25ml of CD8+ T- cells ($@2 \times 10^6$ cell/ml) in each well of a 48-well plate in the presence of 10 ng/ml of IL-7. rHuman IL10 was added the next day at a final concentration of 10 ng/ml and rhuman IL2 was added 48 hours later at 10IU/ml.

Restimulation of the induction cultures with peptide pulsed adherent cells: Seven and fourteen days after the primary induction, the cells were restimulated with peptide-pulsed adherent cells. The PBMCs were thawed and washed twice with RPMI and DNase. The cells were resuspended at 5×10^6 cells/ml and irradiated at ~ 4200 rads. The

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PBMCs were plated at 2×10^6 in 0.5 ml complete medium per well and incubated for 2 hours at 37°C . The plates were washed twice with RPMI by tapping the plate gently to remove the nonadherent cells and the adherent cells were pulsed with $10 \mu\text{g/ml}$ of peptide in the presence of $3 \mu\text{g/ml}$ β_2 microglobulin in 0.25ml RPMI/5%AB per well for 2 hours at 37°C . Peptide solution from each well was aspirated and the wells were washed once with RPMI. Most of the media was aspirated from the induction cultures (CD8+ cells) and brought to 0.5 ml with fresh media. The cells were then transferred to the wells containing the peptide-pulsed adherent cells. Twenty four hours later rhuman IL10 was added at a final concentration of 10 ng/ml and rhuman IL2 was added the next day and again 2-3 days later at 50 IU/ml (V. Tsai, Critical Reviews in Immunology 18(1-2):65-75, 1998). Seven days later the cultures were assayed for CTL activity in a ^{51}Cr release assay. In some experiments the cultures were assayed for peptide-specific recognition in the in situ IFN γ ELISA at the time of the second restimulation followed by assay of endogenous recognition 7 days later. After expansion, activity was measured in both assays for a side by side comparison.

Measurement of CTL lytic activity by ^{51}Cr release.

Seven days after the second restimulation, cytotoxicity was determined in a standard (5 hr) ^{51}Cr release assay by assaying individual wells at a single E:T. Peptide-pulsed targets were prepared by incubating the cells with $10 \mu\text{g/ml}$ peptide overnight at 37°C . Adherent target cells were removed from culture flasks with trypsin-EDTA. Target cells were labelled with $200 \mu\text{Ci}$ of ^{51}Cr sodium chromate (Dupont, Wilmington, DE) for 1 hour at 37°C , washed twice and resuspended at 10^6 per ml and diluted 1:10 with K562 cells (an NK- sensitive erythroblastoma cell line used to reduce non-specific lysis) at a concentration of $3.3 \times 10^6/\text{ml}$. Target cells ($100 \mu\text{l}$) and $100 \mu\text{l}$ of effectors were plated in 96 well round-bottom plates and incubated for 5 hours at 37°C . At that time, $100 \mu\text{l}$ of supernatant were collected from each well and percent lysis was determined according to the formula: [(cpm of the test sample - cpm of the spontaneous ^{51}Cr release

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4µg/ml of diluent (PBS with 3% FCS and 0.05% Tween 20) was added to each well and the plates incubated for 2 hours at room temp.

7. The plates were washed 4x with wash buffer and 100µl/well HRP-streptavidin (Zymed, 43-4323, 1:4000 in diluent) was added and incubated for 1 hour at room temp.
9. The plates were then washed 6x with wash buffer, 100µl/well developing solution (TMB 1:1) was added and developed for about 15 min. The reaction was stopped with 50 ul/well 1M H₃PO₄ and read at OD450.

A culture was considered positive if it measured at least 50 pg of γIFN/well and was twice the background level of expression.

CTL Expansion. Those cultures that demonstrated specific lytic activity against peptide-pulsed targets and/or tumor targets were expanded over a two week period with anti-CD3. Briefly, 5x10⁴ CD8+ cells were added to a T25 flask containing the following: 1x10⁶ irradiated (4,200 rad) PBMC (autologous or allogeneic) per ml, 2x10⁵ irradiated (8,000 rad) EBV- transformed cells per ml, and OKT3 at 30 ng per ml in RPMI-1640 containing 10% (v/v) human ab serum, non-essential AA, sodium pyruvate, 25µM 2-ME, L-glutamine and penicillin/streptomycin. rHuman IL2 was added 24 hours later at a final concentration of 200IU/ml and every 3-4 days thereafter with fresh media at 50 IU/ml. The cells were split if the cell concentration exceeded 1x10⁶/ml and the cultures were assayed between days 13 and 15 at 4 E:T ratios (30, 10, 3 and 1:1) using the same targets as before the expansion.

RESULTS AND DISCUSSION:

1. Peptides identified from wild-type (WT) sequences.

Identification of HLA-A2 supertype degenerate peptides.

Protein sequences from the targeted four tumor antigens (p53, CEA, Her2/neu, and

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Fourteen of these 266 peptides bound A*0201 with high or intermediate affinity and have been tested for A2 crossbinding capacity (NB 903, p. 38). Ten of the 14 peptides tested against A2 supertype molecules bound 3 or more A2 supertype alleles (Table I).

Identification of HLA-A2 restricted CEA-derived epitopes.

Ten peptides have been identified as supertype binders for HLA-A2. Nine peptides were tested in the cellular assay and 6 of these peptides were able to induce a peptide-specific CTL response in at least 2 normal donors except as noted. Previous work done by our group and others demonstrated that additional restimulations were required to detect endogenous recognition when CTLs were induced with CEA peptides. Cultures that lysed peptide-pulsed targets were restimulated twice more before assaying for endogenous recognition. This analysis demonstrated that 5 of these lines also recognized target cells pulsed with the wild-type peptide and tumor targets that endogenously express CEA (Table II).

The epitopes CEA.691 and CEA.605 had been identified previously [I. Kawashima, et al, Human Immunology 59(1):1-14, 1998; E. Ras et al, Human Imm 53:81, 1997; K. Tsang et al, Clin. Cancer Res. 3:2439, 1997, S. Zaremba, et al, Cancer Res. 57:4570, 1997] and so recent efforts have concentrated on CEA.233, CEA.78, CEA.569 and CEA.687. Peptide specific CTLs to CEA.233 (NB 888, p.148), CEA.569 (NB 888, p.179) and CEA. 687 (NB 888, p.171) were observed in one to two donors but endogenous recognition was observed only with CEA.687. Figure 1 shows the 15 wells that demonstrated a positive response to CEA.687 in a ⁵¹Cr release assay.

CTLs from each well were expanded and reassayed against peptide-pulsed and endogenous targets. The results of 4 individual cultures are shown in Figures 2a, 2b, 2c and 2d. As can be seen in 2a through 2c, the peptide-specific CTLs also recognize the endogenous targets. One culture (Fig. 2d) demonstrates significant lysis of peptide-

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pulsed targets but not tumor targets. The CTLs shown in Figures 2a and 2b were also tested against 221A2.1 target cells pulsed with different concentrations of peptide to measure CTL avidity. One line demonstrated high specific lysis down to 1 ng/ml peptide while both exhibited a titration of activity further validating CEA.687 as an epitope (Figure 3).

Even more convincing is the cold target inhibition assay (Figure 4) in which peptide-pulsed targets were incubated with ⁵¹Cr labelled targets to compete for lysis by the CTLs. As can be seen, lysis of radiolabelled targets by two different CTL lines was blocked by increasing numbers of targets pulsed with CEA.687. When an irrelevant peptide was used, in this case HBVc.18, no inhibition of lysis was seen again demonstrating the epitope specificity of these CTLs.

Molecular binding of wild-type p53-derived peptides.

133 sequences carrying the A2 supertype motif were identified within the p53 protein sequence, and all were synthesized. Through the course of previous binding studies, peptides which carry glutamine (Q) at position 2 (L, I, V, M, A, or T at the C-terminus) have also been shown to possess some capacity to bind A2-like molecules. Sixteen Q₂ peptides were also synthesized. Herein, we report that 14 of these 149 peptides bound A*0201 with high or intermediate affinity (NB 903, pp.24, 43).

These 14 peptides were then tested for A2 crossbinding capacity and the results are presented in Table III. Ten highly crossreactive peptides were identified. Although nine of these were disclosed in the C. Melief application (filed internationally May 18, 1993) and by H. Nijman, et al, Immunol. Lett. 40:171, 1994, one WT peptide (position 135, CQLAKTCPV) was not. To avoid any potential issues with the Melief application only the p53.135 peptide was pursued further.

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Cancer,73(1):125, 1997]. MAGE2.157 (M. Visseren, Int. J. Cancer,73(1):125, 1997) and MAGE3.271 have also been identified as epitopes [I. Kawashima, et al, Human Immunology 59(1):1-14, 1998].

Molecular binding of wild-type Her2/neu-derived peptides.

623 WT peptides have been identified within the Her2/neu protein sequence. Of these, 73 scored positive in the A2 algorithm and were synthesized. An additional 90 nonamers and decamers were also synthesized. These 163 were tested for A*0201 binding affinity, with 20 binders being identified (NB 903, p. 40).

All 20 A*0201 binders have been tested for A2 supertype alleles, 9 of them possessing crossbinding capability (Table VII).

Identification of HLA-A2 restricted Her2/neu-derived epitopes.

A total of 9 peptides were identified as candidates for cellular screening by analysis of the Her2/neu sequence. The results of the cellular screening assays are shown in Table VIII [I. Kawashima, et al, Human Immunology 59(1):1-14, 1998]. As can be seen, two peptides, Her2/neu.435 and Her2/neu.369 (B. Fisk et al, J. Exp. Med. 181:2109, 1995), induced CTLs in at least two donors and both recognized tumor targets as well. An example of Her2/neu.369 is shown in figure 7 (NB 888, p. 61). An additional wild-type peptide, Her2/neu.5 was screened on the basis of its A2.1 binding and although it binds to only 2 alleles, it was able to generate a strong CTL response that was both peptide- and tumor-specific.

Additional information on many of the same peptides was generated by a collaborator, Dr. Rolf Kiessling, (Table VIII; Y. Rongcun, Y., et al, Identification of new HER2/neu derived peptide epitopes which can elicit CTL cytotoxic for autologous and allogeneic

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carcinomas, in preparation.). Briefly, PBMCs were isolated from two patients with ovarian cancer, restimulated with peptide-pulsed monocytes and assayed for their ability to recognize peptide-pulsed target cells as well as transfected cells endogenously expressing the antigen. Based on this data, Her2/neu.435 is recognized in 2 donors as well as Her2/neu.369, Her2/neu.952 and Her2/neu.48. Her2/neu.689 is also an epitope but not a supertype binder. Of the other peptides tested, Her2/neu.665 and Her2/neu.773 were recognized by CTLs from only one of the two patients and CTLs to Her2/neu.153 and Her2/neu.789 recognized peptide-pulsed targets alone.

2. Fixed peptides.

Identification of HLA-A2 supertype degenerate fixed peptides.

It has been shown that class I peptide ligands can be modified, or "fixed" to increase their binding affinity and/or degeneracy (Sidney et al, J Immunol 157:3480; Rosenberg, et al, Nature Med. 4:321, 1998). As a supplemental strategy to ensure adequate numbers of degenerate A2 binders for cellular screening, we explored "fixing" peptides at primary anchor positions. Peptides exhibiting at least weak A*0201 binding (IC_{50} of $\leq 5000nM$), and carrying suboptimal anchor residues at either position 2, C termini, or both, can be fixed by introducing canonical substitutions (L at position 2 and V at the C-terminus).

Fixed analog peptides which show at least a three-fold increase in A*0201 binding and bind minimally with an IC_{50} of 500nM, were then tested for A2 crossbinding capacity, along with their wild-type (WT) peptide counterparts. As with WT peptides, fixed analogs must bind at least three of the five A2 supertype alleles to be considered for cellular screening.

Additionally, the selection of analogs for cellular screening was further restricted by their WT parent peptide's capacity to crossbind at least weakly, that is, to have an IC_{50} of

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$\leq 5000\text{nM}$ for 3 or more A2 alleles. The rationale for this requirement is that the WT peptide must be present endogenously in sufficient quantity to be biologically relevant. Fixed peptides have been shown to have increased immunogenicity and cross reactive recognition by T cells specific for the WT epitope (Parkhurst et al, J Immunol 157:2539; Pogue et al, PNAS 92:8166).

Another form of fixing instituted in these studies, unrelated to the primary anchor position, involves the substitution of a cysteine (C) for α -amino butyric acid (B). Due to its chemical nature, cysteine has the propensity to form disulfide bridges and sufficiently alter the peptide structurally as to reduce binding capacity. Substituting B for C not only alleviates this problem, but has been shown to actually improve binding and crossbinding capability in certain instances (Review: A. Sette et al, Persistent Viral Infections, Ed. R. Ahmed and I. Chen, John Wiley & Sons, England).

In the cellular screening of these peptide analogs, it was important to demonstrate that analog-specific CTLs were able to recognize the wild-type peptide and when possible, tumor targets endogenously expressing the epitope.

Molecular binding of CEA fixed peptides.

Sixty-five peptides met the criteria to be fixed by introducing canonical substitutions, showing at least weak A*0201 binding ($\text{IC}_{50} \leq 5000\text{nM}$) and carrying suboptimal anchor residues.

To date, 10 analogs of 9 peptides have been fixed, and both analog and WT versions were tested for crossbinding capacity on other A2 supertype molecules (Table IX).

Eight of the 10 analogs bound minimally 3 of 5 A2 supertype alleles, and their WT versions also bound at least weakly to 3 of 5 alleles (NB 903, p.38). An exception to the

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general strategy was made in the case of the CEA.605 peptide, inasmuch that the fixed analog does not have a three-fold increase in A*0201 binding affinity. This peptide does, however, have increased crossbinding capability, and therefore was included in the list of peptides to be examined in the cellular screening assay.

Cellular screening of CEA analogs.

Eight analogs were identified for cellular screening. One of these, CEA.24V9, was previously identified as an epitope [Kawashima, I. et al, Human Immunology 1998; Jan: 59(1):1-14]. Three additional peptides were screened and, as shown in Table X, CEA.233V10 (NB 888, pp. 60, 101 and 148), CEA.605V9 (NB 888, p. 148) and CEA.589V9 (NB 888, p. 162) all induced CTL that were able to recognize peptide-pulsed and/or tumor targets after 2 and 4 restimulations respectively. Figures 8a and 9a are examples of the peptide specific lysis seen with peptides CEA.233V10 and CEA.605V9 respectively. After expansion of the positive cultures, the CTLs were again tested against the analog and also the parental peptide and tumor targets (Figures 8b and 9b). CTLs to both analogs demonstrated recognition of the wildtype peptide and the tumor line, KATO III. In addition to being immunogenic, CEA.233V10 and CEA.605V9 showed improved overall binding when compared to the corresponding wildtype peptide as well as supertype binding to 4 alleles. An additional epitope, CEA.589V9, was immunogenic and CEA.589V9-specific CTLs recognized the wildtype peptide (Figures 10a, 10b and 10c) but endogenous recognition was not observed.

Molecular binding of p53 fixed peptides.

Nineteen peptides meeting the prescribed parameters were fixed (NB 903, p. 25). The results are shown in Table XI.

Eighteen analog peptides which meet the prescribed criteria were tested for crossbinding capacity on other A2 supertype molecules (Table XII). Eleven of these analogs exhibited

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improved crossbinding capability (NB 903, p. 57).

Cellular screening of p53 analogs.

Eleven analogs have been identified as HLA-A2 supertype binding peptides (Table XIII) and cellular screening was done with all 11 peptides. In the case of p53, it was important to demonstrate induction of peptide-specific CTL and then to use those cells to identify an endogenous tumor target. Each assay also included the epitope HBVc.18 as an internal control. When peptide p53.139L2 was used to induce CTLs in a normal donor, measurable CTL activity was observed in 3 of 48 wells (Figure 11a, NB 888, p. 79). Each well was expanded and two weeks later reassayed against the induction peptide and the appropriate wildtype peptide. As can be seen, p53.139L2-specific CTLs expanded well and maintained their lytic activity. Additionally, two of these cultures recognized the parental, wildtype peptide (Figures 11b and 11c, NB 888, p. 139).

These cells were then used to assess endogenous target cell lines. Numerous HLA-A2+, p53 expressing tumor lines have been described in the literature (W. Storkus, personal communication; M. Theobald et al, PNAS 92: 11993, 1995) and were readily available. These included MCF-7, a breast tumor line; BT549, breast, infiltrating ductal carcinoma and SW480, colon adenocarcinoma (PNAS 1995, 92: 11993). Saos-2, an osteogenic sarcoma which is HLA-A2+ and p53- was used as the negative endogenous target. Figures 12a and 12b show that two individual CTL cultures to peptide p53.139L2 demonstrated significant lysis of the endogenous target BT549 (NB888, p. 139). This was the target line used for all subsequent assays. We were also able to obtain the Saos-2/175 line (Saos-2 transfected with the p53 gene) at a later time and this line was also included as a tumor target as noted.

Of the available analogs tested, ten induced a peptide-specific response in 2 or more donors. Of these 10, eight generated CTLs that recognized the wild-type peptide and 4 of

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these recognized tumor targets (Table XIII). Two of these analogs, p53.139L2 and p53.139L2B3, differ only at position three. Figures 13a and 13b (NB 888, p.140) show the assay results of CTLs to p53.139L2B3 tested against peptide-pulsed and tumor targets. As can be seen in Figure 13b, these CTLs recognize the wild-type peptide as well as the analog and tumor target BT549. p53.149M2, an analog that represents a significant improvement over the wildtype peptide is shown in Figures 14a and 14b (NB 888, p. 152). Six individual wells met the criteria for positivity and one well maintained that activity upon expansion. All the CTLs generated recognized the wildtype peptide and were also able to lyse the Saos-2/175 transfected line. A fourth epitope, p53.69L2V8, also demonstrated recognition of the wildtype peptide (data not shown, NB 888, p. 151).

Molecular binding of MAGE2/3 fixed peptides.

Of the 19 MAGE2/3-derived A*0201 binders, 14 carry suboptimal anchors and are candidates for anchor fixing. To date, 5 analogs of two WT peptides have been synthesized and tested for A2 supertype molecules (NB 903, p. 58; Table XIV).

MAGE3.112 analogs exhibit increased A*0201 binding affinity, but the parent peptide already binds all 5 A2 supertype alleles, and significant improvement in this area was not achieved. The MAGE3.220 analog, however, does meet all fixed analog criteria, having increased A*0201 binding affinity three-fold and improved crossbinding.

Additionally, 24 of 26 weak binding A*0201 peptides are also candidates.

Cellular screening of MAGE2/3 analogs.

No cellular screening was done because the analogs provided no improvement over the wild-type.

Molecular binding of Her2/neu fixed peptides.

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Of the 20 A*0201 binders, 15 carry suboptimal anchors, and are candidates for fixing. To date, 8 analogs of 6 A*0201 binders have been fixed (NB 903, p.58), and tested for primary and supertype binding (Table XV). In five of 6 cases, A*0201 binding was improved at least three-fold. In three cases, crossbinding capability was also improved. In one special case, peptide Her2/neu.153 did not have a three-fold increase in A*0201 binding, but crossbinding capability was improved.

Additionally, 22 weak binding A*0201 peptides carrying suboptimal anchors were also identified, and can be fixed if desired.

Cellular screening of Her2/neu analogs.

Two analogs of Her2/neu.5 and 1 version of Her2/neu.952 were screened in the primary CTL induction assay. Her2/neu.5B3V9 (NB 865, pp. 148, 168) and Her2/neu.952L2B7V10 (NB 865, pp. 143, 148, 170) induced peptide-specific CTLs in at least 2 donors (data not shown) but when the positive cultures were expanded, no wild-type peptide or endogenous recognition was observed (Table XVI).

CONCLUSION:

For each of the four tumor antigens CEA, p53, Her2/neu, and MAGE2/3, at least 2 peptides were identified that satisfy each of the parameters that define an epitope: 1) they are degenerate A2 supertype binders, possessing $IC_{50}s \leq 500nM$ for three of the five A2 supertype alleles tested, 2) they elicit a peptide-specific CTL response and 3) they recognize the wild-type peptide and/or tumor targets.

The HLA-A2 restricted epitopes identified by antigen are as follows:

CEA: CEA687, and CEA691.

MAGE2/3: MAGE2.157, MAGE3.112, and MAGE3.160.

Her2/neu: Her2/neu.369 and Her2/neu.435.

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Figure legends

Figure 1a. Recognition of CEA.687 peptide-pulsed targets by peptide specific CTLs. CTLs from individual wells were screened against 221A2.1 without peptide (hatched bars) or 221A2.1 pulsed overnight with 10 ug/ml peptide (solid bars). K562 cells were added to reduce lysis by natural killer (NK) cells.

Figures 2a-2d. Expanded CEA.687 specific CTLs from wells 15, 23, 42, and 41 respectively recognize peptide-pulsed and tumor targets. The CTLs were tested against 221A2.1 cells (open circle), 221A2.1 pulsed with CEA.687 (closed circle), HT29, the negative endogenous target (open square) and SW403, the CEA positive tumor target (closed square).

Figure 3. CEA.687 peptide concentration affects lysis of target cells by peptide-specific CTLs. Expanded CEA.687 specific CTLs from wells 15 (open circles) and 23 (closed squares) were tested against 221A2.1 target cells pulsed overnight with varying amounts of peptide.

Figure 4. Lysis of radiolabelled endogenous targets by CEA.687 specific CTLs is inhibited by increasing numbers of peptide-pulsed 221A2.1 targets. Two CTL lines at 1×10^5 cells per well were incubated for 5 hours with 1×10^4 ^{51}Cr labelled SW403 cells/well and varying numbers of 221A2.1 cells pulsed overnight with 10 ug/ml CEA.687 peptide (line 15, closed circle; line 42, closed square) or a nonspecific peptide, in this case HBV.18 (line 15, open circle; line 42, open square).

Figures 5a and 5b. Recognition of MAGE3.159 peptide-pulsed targets and tumor targets by peptide specific CTLs in the IFN γ ELISA. Figure 5a shows CTLs from individual wells screened against 221A2.1 without peptide (hatched bars) or 221A2.1 pulsed

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overnight with 10 ug/ml peptide (solid bars). Figure 5b represents CTL recognition of 888mel (the negative, HLA mismatched tumor line; hatched bars) and 624mel, the positive tumor target line (solid bars).

Figures 6a and 6b. Recognition of MAGE3.160 peptide-pulsed targets and tumor targets by peptide specific CTLs in the IFN γ ELISA. Figure 6a shows CTLs from individual wells screened against 221A2.1 without peptide (hatched bars) or 221A2.1 pulsed overnight with 10 ug/ml peptide (solid bars). Figure 6b represents CTL recognition of 888mel (the negative, HLA mismatched tumor line; hatched bars) and 624mel, the positive tumor target line (solid bars).

Figure 7. Lysis of HER2/neu.369 peptide-pulsed targets and tumor targets by peptide specific CTLs. CTLs from one well of the primary induction cultures were tested against 221A2.1 (open bar), 221A2.1 pulsed with 10 ug/ml peptide overnight (black bar), HT29 (negative tumor targets, light gray bar) and SW403 (positive tumor targets, dark gray bar).

Figure 8a. CEA. 233V10 induced CTL are able to recognize peptide-pulsed 221A2.1 cells. CTLs were tested against 221A2.1 cells (hatched bars) and 221A2.1 peptide-pulsed cells (black bars).

Figure 8b. Expanded CEA. 233V10-specific CTLs from wells 4 and 10 were able to recognize the wildtype peptide and tumor targets as well as the analog. The CTLs were tested against 221A2.1 cells (hatched bars), 221A2.1 pulsed with CEA.233V10 (black bar), 221A2.1 pulsed with CEA.233 (crosshatched bar), HT29 (hatched), SW403 (white bar) and KATO III, a second CEA positive, A2 positive tumor target (gray bar).

Figure 9a. Recognition of peptide-pulsed target cells by CEA. 605V9 specific CTL. CTLs

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from a primary induction with peptide CEA. 605V9 were tested for lytic activity against 221A2.1 without peptide (hatched bar) or 221A2.1 pulsed overnight with 10 ug/ml peptide (black bar). K562 cells were added to reduce lysis by natural killer (NK) cells.

Figure 9b. Expanded CEA.605V9 CTLs from wells 23 and 39 respectively recognize the wildtype peptide and tumor cells as well as the analog. The CTLs were tested in the IFN γ ELISA against 221A2.1 cells (hatched bars), 221A2.1 pulsed with CEA.605V9 (black bar), 221A2.1 pulsed with CEA.605 (crosshatched bar), HT29 (hatched), SW403 (white bar) and KATO III, a second CEA positive, A2 positive tumor target (gray bar).

Figure 10a. Recognition of peptide-pulsed target cells by CEA. 589V9 specific CTL. CTL from a primary induction with peptide CEA. 589V9 were tested for lytic activity against 221A2.1 without peptide (hatched bar) or 221A2.1 pulsed overnight with 10 ug/ml peptide (black bar). K562 cells were added to reduce lysis by NK cells.

Figures 10b and 10c. The expanded CTLs to CEA.589V9 (from wells 13 and 23 respectively) recognize the wildtype peptide. The CTLs were tested against 221A2.1 cells (open circle), 221A2.1 pulsed with CEA.589V9 (closed circle) and CEA.589 (open triangle).

Figure 11a. Peptide p53.139L2 induced CTL that recognized peptide-pulsed target cells. CTLs from individual wells were screened against 221A2.1 without peptide (hatched bars) or 221A2.1 pulsed overnight with 10 ug/ml peptide (solid bars). K562 cells were added to reduce lysis by NK cells.

Figures 11b and 11c. Expanded p53.139L2-specific CTL from wells 41 and 48 respectively, recognize the wildtype peptide. The targets were 221A2.1 cells (open circle), 221A2.1 pulsed with p53.139L2 (closed circle) and p53.139 (open triangle).

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Figures 12a and 12b. Expanded p53.139L2-specific CTL, from wells 41 and 48 respectively, also recognize endogenously expressed p53. The cell lines tested were 221A2.1 cells (open circle), Saos-2 (open triangle), MCF-7 (closed triangle), SW480 (closed circle), and BT549 (closed square).

Figure 13a. CTLs induced with p53.139L2B3 peptide recognize peptide-pulsed 221A2.1 targets. CTLs from individual wells were screened against 221A2.1 without peptide (hatched bars) or 221A2.1 pulsed overnight with 10 ug/ml peptide (solid bars). K562 cells were added to reduce lysis by NK cells.

Figure 13b. Expanded p53.139L2B3 CTLs from well 42 recognize the wildtype peptide and tumor cells as well as the analog. The CTLs were tested in the IFN γ ELISA against 221A2.1 cells (hatched bars), 221A2.1 pulsed with p53.139L2B3 (black bar), 221A2.1 pulsed with p53.139 (crosshatched bar), Saos-2 (hatched) and BT549, a p53 positive, A2 positive tumor target (gray bar).

Figure 14a. CTLs induced with p53.149M2 peptide recognize 221A2.1 targets pulsed with the analog and wildtype peptides. CTLs from individual wells were screened against 221A2.1 without peptide (hatched bars) or 221A2.1 pulsed overnight with 10 ug/ml p53.149M2 (black bars) or p53.149. K562 cells were added to reduce lysis by NK cells.

Figure 14b. Expanded p53.149M2 CTLs from well 40 recognize the wildtype peptide and tumor cells as well as the analog. The CTLs were tested in the ^{51}Cr release assay against 221A2.1 cells (open circles), 221A2.1 pulsed with p53.149M2 (closed circles), 221A2.1 pulsed with p53.149 (open triangles), Saos-2, the p53 negative line (open squares) and Saos-2/175, a p53 positive, A2 positive transfected tumor target (closed squares).

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Table I. Crossbinding data of 14 wild type CEA A*0201 binders.

| Source | AA | Sequence | A*0201 nM | A*0202 nM | A*0203 nM | A*0206 nM | A*6802 nM | No. A2 Alleles Crossbound |
|---------|----|------------|--------------|--------------|--------------|--------------|--------------|---------------------------------|
| CEA.24 | 9 | LTFWNPPT | 179 | 1720 | 67 | 755 | -- | 2 |
| CEA.78 | 9 | QIGYVIGT | 313 | 148 | 106 | 100 | 150 | 5 |
| CEA.233 | 10 | VLYGPDAPTI | 128 | 606 | 270 | 804 | -- | 2 |
| CEA.354 | 10 | YLWWVNNQSL | 26 | 108 | 26 | 487 | 67 | 5 |
| CEA.411 | 10 | VLYGPDPTI | 294 | 358 | 476 | 7400 | -- | 3 |
| CEA.432 | 9 | NLSLSCHAA | 455 | 2867 | 1449 | 18500 | -- | 1 |
| CEA.532 | 10 | YLWWVNNQSL | 33 | 331 | 21 | 2056 | 286 | 4 |
| CEA.569 | 9 | YVCGIQNSV | 98 | 358 | 159 | 80 | 181 | 5 |
| CEA.589 | 9 | VLYGPDPTI | 200 | 878 | 53 | 638 | -- | 2 |
| CEA.605 | 9 | YLSGANLNL | 28 | 165 | 2.4 | 804 | -- | 3 |
| CEA.687 | 9 | ATVGIMGV | 36 | 8.8 | 20 | 11 | 0.80 | 5 |
| CEA.690 | 10 | GIMGVLVGV | 64 | 205 | 31 | 142 | 500 | 5 |
| CEA.691 | 9 | IMIGVLVGV | 69 | 62 | 13 | 106 | 89 | 5 |
| CEA.691 | 10 | IMIGVLVGV | 227 | 68 | 44 | 726 | 1509 | 3 |

-- indicates binding affinity $\geq 10,000$ nM.

Table II. Immunogenicity of 6 wild type CEA crossbinders.

| Source | AA | Sequence | A*0201 nM | A*0202 nM | A*0203 nM | A*0206 nM | A*6802 nM | No. A2 Alleles Crossbound | CTL Wild-type ¹ | CTL Tumor |
|---------|----|------------------------|--------------|--------------|--------------|--------------|----------------|---------------------------------|-------------------------------|--------------|
| CEA.78 | 9 | QIIQYVIGT | 313 | 148 | 106 | 100 | 151 | 5 | 0/3 | |
| CEA.354 | 10 | YLWWVNNQSL | 26 | 108 | 26 | 487 | 333 | 5 | 1/2 | 0/1 |
| CEA.569 | 9 | YVCGIQNSV ² | 98 | 358 | 159 | 80 | 182 | 5 | 1/2 | 0/1 |
| CEA.605 | 9 | YLSGANLNL | 28 | 165 | 2.4 | 804 | — ² | 3 | 2/2 | 1/2 |
| CEA.687 | 9 | ATVGMIGV | 36 | 8.8 | 20 | 11 | 0.80 | 5 | 1/1 | 1/1 |
| CEA.691 | 9 | IMGVLVGV | 69 | 62 | 13 | 106 | 89 | 5 | 8/8 | 4/7 |

1) Number of donors yielding a positive response/total tested.

2) — indicates binding affinity $\geq 10,000$ nM.

Table III. Crossbinding data of 14 wild type p53 A*0201 binders.

| Source | AA | Disclosed | Sequence | A*0201 nM | A*0202 nM | A*0203 nM | A*0206 nM | A*6802 nM | No. A2 Alleles Crossbound |
|---------|----|----------------|-------------|--------------|--------------|--------------|--------------|--------------|---------------------------------|
| p53.24 | 9 | Melief | KLIPENNVL | 313 | 1955 | -- | 1194 | -- | 1 |
| p53.25 | 11 | Sherman | LLPENNVLSPL | 19 | 6.2 | 4.5 | 12 | 1702 | 4 |
| p53.65 | 10 | Melief | RMPEAAPVA | 78 | 102 | 13 | 841 | -- | 3 |
| p53.65 | 9 | Melief | RMPEAAPV | 119 | 23 | 22 | 70 | -- | 4 |
| p53.113 | 10 | Melief | FLHSGTAKSV | 357 | 179 | 15 | 4625 | -- | 3 |
| p53.132 | 9 | Melief | KMFCQLAKT | 333 | 33 | 18 | 106 | -- | 4 |
| p53.135 | 9 | | QLAKTCPV | 208 | 43 | 143 | 90 | -- | 4 |
| p53.136 | 8 | | QLAKTCPV | 455 | -- | 100 | 2643 | 1067 | 2 |
| p53.164 | 9 | Melief | KOSQHMTEV | 500 | 130 | 170 | 285 | -- | 4 |
| p53.187 | 11 | Melief/Sherman | GLAPPQHLRV | 79 | 39 | 11 | 55 | -- | 4 |
| p53.193 | 11 | Melief | HLIRVEGNLRV | 385 | 1387 | 83 | 1194 | 1778 | 2 |
| p53.229 | 9 | Sherman | CTTHYNVM | 278 | 287 | 2564 | 561 | 181 | 3 |
| p53.263 | 10 | Melief | NLGRNSFEV | 217 | -- | 2500 | 881 | -- | 1 |
| p53.264 | 9 | Melief | LLGRNSFEV | 85 | 358 | 37 | 206 | -- | 4 |

-- indicates binding affinity $\geq 10,000$ nM.

Table IV. Immunogenicity of a wild-type p53 crossbinder.

| Source | Sequence | A*0201 nM | A*0202 nM | A*0203 nM | A*0206 nM | A*6802 nM | No. A2 Alleles Crossbound | CTL Wild-type ¹ | CTL Tumor |
|---------|-----------|--------------|--------------|--------------|--------------|----------------|---------------------------------|-------------------------------|--------------|
| p53.135 | COLAKTCPV | 208 | 43 | 143 | 90 | — ² | 4 | 1/4 | 0/1 |

1) Number of donors yielding a positive response/total tested.

2) — indicates binding affinity $\geq 10,000$ nM.

Table V. Crossbinding data of 19 wild-type MAGE2/3 A*0201 binders.

| Source | AA | Sequence | A*0201 nM | A*0202 nM | A*0203 nM | A*0206 nM | A*6802 nM | No. A2 Alleles Crossbound |
|------------|----|-------------|--------------|--------------|--------------|--------------|--------------|---------------------------------|
| MAGE2.112 | 9 | KMVELVHFL | 38 | 15 | 9.1 | 49 | 364 | 5 |
| MAGE2.112 | 10 | KMVELVHFL | 23 | 39 | 127 | 9.0 | 2667 | 4 |
| MAGE2.112 | 11 | KMVELVHFL | 5.0 | 45 | 63 | 109 | 7692 | 4 |
| MAGE2.153 | 9 | KASEYLQLV | 152 | 116 | 17 | 185 | 4878 | 4 |
| MAGE2.157 | 10 | YLQLVFGIEV | 50 | 165 | 345 | 370 | 9302 | 4 |
| MAGE2.160 | 10 | LVFGIEVVEV | 357 | 21 | 44 | 29 | 8.0 | 5 |
| MAGE2.220 | 9 | KIWEELSM | 167 | 642 | 175 | 29 | -- | 3 |
| MAGE2.271 | 9 | FLWCPRALI | 238 | 96 | 137 | 1542 | 95 | 4 |
| MAGE2.277 | 10 | ALIEISVVKV | 500 | 729 | 125 | 1947 | 3077 | 2 |
| MAGE2/3.44 | 10 | TLVEVTLGEV | 67 | 39 | 4.3 | 218 | 33 | 5 |
| MAGE3.112 | 9 | KVAELVHFL | 68 | 29 | 14 | 168 | 17 | 5 |
| MAGE3.112 | 10 | KVAELVHFL | 54 | 36 | 217 | 206 | 11 | 5 |
| MAGE3.159 | 11 | QLVFGIELMEV | 7.9 | 74 | 217 | 185 | 267 | 5 |
| MAGE3.160 | 10 | LVFGIELMEV | 29 | 20 | 7.7 | 29 | 14 | 5 |
| MAGE3.174 | 11 | HLVFATCLGL | 56 | 741 | 769 | -- | 4494 | 1 |
| MAGE3.176 | 9 | YVFATCLGL | 185 | 45 | 37 | 1028 | 222 | 4 |
| MAGE3.195 | 11 | IMPKAGLLIV | 20 | 226 | 15 | 176 | -- | 4 |
| MAGE3.220 | 9 | KIWEELSVL | 333 | 391 | 2381 | 308 | -- | 3 |
| MAGE3.271 | 9 | FLWCPRALV | 31 | 43 | 14 | 336 | 40 | 5 |

-- indicates binding affinity $\geq 10,000$ nM.

Table VI. Immunogenicity of 13 wild-type MAGE2/3 crossbinders.

| Source | AA | Sequence | A*0201 nM | A*0202 nM | A*0203 nM | A*0206 nM | A*6802 nM | No. A2 Alleles Crossbound | CTL Wild-type ¹ | CTL Tumor |
|-----------|----|-------------|--------------|--------------|--------------|--------------|-----------------|---------------------------------|-------------------------------|------------------|
| MAGE2.112 | 9 | KMVELVHFL | 9.8 | 25 | 17 | 123 | 2353 | 4 | 1/1 | 0/1 |
| MAGE2.112 | 10 | KMVELVHFL | 23 | 39 | 127 | 9.0 | 2667 | 4 | 1/1 | 0/1 |
| MAGE2.112 | 11 | KMVELVHFL | 5.0 | 45 | 63 | 109 | 7692 | 4 | 1/1 | 0/1 |
| MAGE2.153 | 9 | KASEYLQLV | 152 | 116 | 17 | 185 | 4878 | 4 | 2/4 | 0/2 |
| MAGE2.157 | 10 | YLQLVFGIEV | 50 | 165 | 345 | 370 | 9302 | 4 | 3/3 | 1/3 |
| MAGE2.160 | 10 | LVFGIEVVEV | 357 | 20 | 43 | 28 | 8.0 | 5 | 4/4 | 0/3 |
| MAGE3.112 | 9 | KVAELVHFL | 68 | 29 | 14 | 168 | 17 | 5 | 3/4 | 3/4 |
| MAGE3.112 | 10 | KVAELVHFL | 54 | 36 | 217 | 206 | 11 | 5 | 0/1 | 0/1 |
| MAGE3.159 | 11 | QLVFGIELMEV | 7.9 | 74 | 217 | 185 | 267 | 5 | 3/3 | 1/3 ² |
| MAGE3.160 | 10 | LVFGIELMEV | 29 | 20 | 7.7 | 28 | 14 | 5 | 4/4 | 1/4 ² |
| MAGE3.195 | 11 | IMPKAGLLIV | 20 | 226 | 14 | 176 | -- ³ | 4 | 3/4 | 0/3 |
| MAGE3.220 | 9 | KIWEELSVL | 357 | 391 | 2381 | 308 | -- | 3 | 3/4 | 0/3 |
| MAGE3.271 | 9 | FLWGPRLV | 31 | 43 | 14 | 336 | 40 | 5 | 4/4 | 2/4 |

- 1) Indicates the number of donors positive over the total number of donors tested.
- 2) A positive result was seen after the second restim.
- 3) -- indicates binding affinity $\geq 10,000$ nM.

Table VII. Crossbinding data of 20 wild-type Her2/neu A*0201 binders.

| Source | AA | Sequence | A*0201 nM | A*0202 nM | A*0203 nM | A*0206 nM | A*6802 nM | No. A2 Alleles Crossbound |
|--------------|----|------------|--------------|--------------|--------------|--------------|--------------|---------------------------------|
| Her2/neu.5 | 9 | ALCRWGILL | 100 | -- | 278 | -- | -- | 2 |
| Her2/neu.5 | 10 | ALCRWGILLA | 139 | 1955 | 12 | 1947 | 2500 | 2 |
| Her2/neu.48 | 9 | HLVQGCQVY | 139 | 307 | 13 | 514 | 1143 | 3 |
| Her2/neu.106 | 9 | QLFEDNYAL | 17 | 226 | 11 | 463 | 2105 | 4 |
| Her2/neu.106 | 10 | QLFEDNYALA | 357 | 662 | 9.1 | 218 | 74 | 4 |
| Her2/neu.144 | 10 | SLTEILKGGV | 238 | -- | 22 | -- | -- | 2 |
| Her2/neu.153 | 9 | VLIQRNPOL | 23 | 3909 | 3.3 | 1057 | -- | 2 |
| Her2/neu.369 | 9 | KIFGSLAFL | 36 | 9.0 | 19 | 23 | 3333 | 4 |
| Her2/neu.435 | 9 | ILHNGAYSL | 75 | 358 | 100 | 569 | -- | 3 |
| Her2/neu.466 | 9 | ALIHNTL | 278 | 1265 | 10 | 1762 | -- | 2 |
| Her2/neu.508 | 9 | GLACHQLCA | 417 | -- | 127 | -- | 9091 | 2 |
| Her2/neu.653 | 9 | SISAVVGI | 69 | 524 | 35 | 285 | 148 | 4 |
| Her2/neu.665 | 9 | VVLGVVFGI | 14 | -- | 2500 | 430 | 2000 | 2 |
| Her2/neu.689 | 9 | RLLQETELV | 21 | -- | 625 | 34 | -- | 2 |
| Her2/neu.767 | 9 | ILDEAVVMA | 238 | -- | 4167 | 3083 | -- | 1 |
| Her2/neu.773 | 10 | VMAGVGSPYV | 200 | 391 | 13 | 3700 | -- | 3 |
| Her2/neu.789 | 9 | CLTSTVQLV | 208 | 457 | 6.7 | 308 | 8000 | 4 |
| Her2/neu.799 | 9 | QLMPYGCCL | 217 | 977 | 114 | 712 | -- | 2 |
| Her2/neu.952 | 10 | YMMVVKCWM | 20 | 307 | 83 | 116 | 267 | 5 |
| Her2/neu.952 | 9 | YMMVVKCWM | 217 | -- | 625 | 2643 | 1000 | 1 |

-- indicates binding affinity $\geq 10,000$ nM.

Table VIII. Immunogenicity of 10 wild-type Her2/neu peptides.

| Source | AA | Sequence | A*0201 nM | A*0202 nM | A*0203 nM | A*0206 nM | A*6802 nM | No. A2 Alleles Crossbound | CTL Wild-type ¹ | CTL Tumor ¹ | CTL Wild-type ² | CTL Tumor ² |
|--------------|----|------------|--------------|----------------|--------------|--------------|--------------|---------------------------------|-------------------------------|---------------------------|-------------------------------|---------------------------|
| Her2/neu.5 | 9 | ALCRWGILL | 100 | — ³ | 278 | — | — | 2 | 2/2 | 2/2 | | |
| Her2/neu.48 | 9 | HLVQCCQV | 139 | 307 | 13 | 514 | 1143 | 3 | 1/2 | 0/2 | 2/2 | 1/2 |
| Her2/neu.106 | 9 | QLFEDNYAL | 17 | 226 | 11 | 463 | 2105 | 4 | 0/2 | 0/2 | | |
| Her2/neu.106 | 10 | QLFEDNYALA | 357 | 662 | 9.1 | 218 | 74 | 4 | 0/2 | 0/2 | | |
| Her2/neu.369 | 9 | KIRGSLAFL | 36 | 9.0 | 19 | 23 | 3333 | 4 | 6/7 | 4/7 | 2/2 | 2/2 |
| Her2/neu.435 | 9 | ILHNGAYSL | 75 | 358 | 100 | 569 | — | 3 | 3/3 | 1/3 | 2/2 | 2/2 |
| Her2/neu.653 | 9 | SISAVVGI | 69 | 524 | 35 | 285 | 148 | 4 | 0/3 | | | |
| Her2/neu.773 | 10 | VMAGVGSFV | 200 | 391 | 13 | 3700 | — | 3 | 1/2 | 0/2 | 1/2 | 1/2 |
| Her2/neu.789 | 9 | CLTSTVQLV | 208 | 457 | 6.7 | 308 | 8000 | 4 | 1/4 | 0/4 | 1/2 | |
| Her2/neu.952 | 10 | YIMVKKCWMI | 20 | 307 | 83 | 116 | 267 | 5 | 0/1 | 0/1 | 2/2 | 2/2 |

1) Number of donors yielding a positive response/total tested.

2) Kiessling data from ovarian cancer patients.

3) — indicates binding affinity $\geq 10,000$ nM.

Table IX. Crossbinding data of 9 fixed CEA peptides.¹

| Source | AA | Sequence | A*0201 nM | A*0202 nM | A*0203 nM | A*0206 nM | A*6802 nM | No. A2 Alleles Crossbound |
|------------|----|-----------|--------------|--------------|--------------|--------------|-----------------|---------------------------------|
| CEA.24 | 9 | LTFTWNPPT | 179 | 1720 | 67 | 755 | -- ² | 2 |
| CEA.24M2V9 | 9 | LMFTWNPV | 4.5 | 782 | 7.7 | 34 | 3333 | 3 |
| CEA.24V9 | 9 | LTFTWNPV | 16 | 307 | 26 | 56 | 952 | 4 |
| CEA.78 | 9 | QHGVICT | 313 | 148 | 106 | 100 | 150 | 5 |
| CEA.78L2V9 | 9 | QLGVIGV | 9.4 | 5.9 | 2.3 | 21 | 2.3 | 5 |
| CEA.233 | 10 | VLGPDAPTI | 128 | 606 | 270 | 804 | -- | 2 |
| CEA.233V10 | 10 | VLGPDAPTV | 26 | 430 | 16 | 206 | 952 | 4 |
| CEA.411 | 10 | VLGPDPTI | 294 | 358 | 476 | 7400 | -- | 3 |
| CEA.411V10 | 10 | VLGPDPTV | 161 | 105 | 91 | 2467 | -- | 3 |
| CEA.569 | 9 | VVCGIONSV | 98 | 358 | 159 | 80 | 181 | 5 |
| CEA.569L2 | 9 | YLCGIQNSV | 50 | 24 | 12 | 31 | 3478 | 4 |
| CEA.589 | 9 | VLGPDPI | 200 | 878 | 53 | 638 | -- | 2 |
| CEA.589V9 | 9 | VLGPDPTV | 20 | 165 | 91 | 154 | 9756 | 4 |
| CEA.605 | 9 | YLSGANLNL | 28 | 165 | 2.4 | 804 | -- | 3 |
| CEA.605V9 | 9 | YLSGANLNV | 73 | 13 | 13 | 80 | 1600 | 4 |
| CEA.687 | 9 | ATVGIMGV | 36 | 8.8 | 20 | 11 | 0.80 | 5 |
| CEA.687L2 | 9 | ALVGIMGV | 10 | 63 | 31 | 100 | 102 | 5 |
| CEA.691 | 9 | IMGVLGV | 69 | 62 | 13 | 106 | 89 | 5 |
| CEA.691L2 | 9 | ILGVLGV | 22 | 8.0 | 3.2 | 16 | 160 | 5 |

1) Wild-type peptides presented for reference purposes.

2) -- indicates binding affinity $\geq 10,000$ nM.

Table X. Immunogenicity of 4 fixed CEA crossbinders.

| Source | AA | Sequence | A*0201 nM | A*0202 nM | A*0203 nM | A*0206 nM | A*6802 nM | No. A2 Alleles Crossbound | CTL Peptide ¹ | CTL Wild-type | CTL Tumor |
|------------|----|-----------|--------------|--------------|--------------|--------------|----------------|---------------------------------|-----------------------------|------------------|--------------|
| CEA.24 | 9 | LTFWNPPT | 179 | 1720 | 67 | 755 | — ² | 2 | | 0/1 | 0/1 |
| CEA.24V9 | 9 | LTFWNPPV | 16 | 307 | 26 | 56 | 952 | 4 | 1/1 | | 1/1 |
| CEA.233 | 10 | VLGPDAPTI | 128 | 606 | 270 | 804 | — | 2 | | 2/4 | 0/3 |
| CEA.233V10 | 10 | VLGPDAPTV | 26 | 430 | 16 | 206 | 952 | 4 | 3/4 | 2/2 | 1/4 |
| CEA.589 | 9 | VLGPDITI | 200 | 878 | 53 | 638 | — | 2 | | 1/1 | 0/1 |
| CEA.589V9 | 9 | VLGPDITPV | 20 | 165 | 91 | 154 | 9756 | 4 | 2/2 | 2/2 | 0/2 |
| CEA.605 | 9 | YISGANLNL | 28 | 165 | 2.4 | 804 | — | 3 | | 2/2 | 1/2 |
| CEA.605V9 | 9 | YISGANLNV | 73 | 13 | 13 | 80 | 1600 | 4 | 4/4 | 3/4 | 1/4 |

1) Number of donors yielding a positive response/total tested.

2) — indicates binding affinity $\geq 10,000$ nM.

Table XI. A*0201 binding of 20 fixed p53 peptides.

| Source | AA | Sequence | A*0201 nM |
|---------------|----|-------------|--------------|
| p53.24 | 9 | KLLPENNVL | 313 |
| p53.24V9 | 9 | KLLPENNVV | 385 |
| p53.25 | 11 | LLPENNVLSPL | 19 |
| p53.25V9 | 11 | LLPENNVLSPV | 39 |
| p53.65 | 9 | RMPEAAPPV | 119 |
| p53.65L2 | 9 | RLPEAAPPV | 78 |
| p53.65 | 10 | RMPEAAPVA | 78 |
| p53.65L2V10 | 10 | RLPEAAPVV | 143 |
| p53.65M2V10 | 10 | RMPEAAPVV | 54 |
| p53.69 | 8 | AAPPVAPA | 5000 |
| p53.69L2V8 | 8 | ALPPVAPV | 217 |
| p53.101 | 11 | KTYQGSYGFR | 1786 |
| p53.101L2V11 | 11 | KLYQGSYGFRV | 81 |
| p53.113 | 11 | FLHSGTAKSVT | 5000 |
| p53.113V11 | 11 | FLHSGTAKSVV | 1220 |
| p53.129 | 9 | ALNKMFCQL | 735 |
| p53.129V9 | 9 | ALNKMFCQV | 75 |
| p53.129B7V9 | 9 | ALNKMFBQV | 192 |
| p53.129 | 10 | ALNKMFCQLA | 1316 |
| p53.129V10 | 10 | ALNKMFCQLV | 217 |
| p53.132 | 9 | KMFCQLAKT | 333 |
| p53.132V9 | 9 | KMFCQLAKV | 33 |
| p53.132B4V9 | 9 | KMFBQLAKV | 125 |
| p53.132L2V9 | 9 | KLFCQLAKV | 98 |
| p53.135 | 9 | CQLAKTCPV | 208 |
| p53.135L2 | 9 | CLLAKTCPV | 125 |
| p53.135B1B7 | 9 | BQLAKTBPV | 102 |
| p53.135B1L2B7 | 9 | BLLAKTBPV | 46 |
| p53.139 | 9 | KTCPVQLWV | 725 |
| p53.139L2 | 9 | KLCPVQLWV | 122 |
| p53.139L2B3 | 9 | KLBPVQLWV | 46 |
| p53.149 | 9 | STPPPGTRV | 909 |
| p53.149M2 | 9 | SMPPPGTRV | 172 |
| p53.149L2 | 9 | SLPPPGTRV | 122 |
| p53.164 | 9 | KQSQHMTEV | 500 |
| p53.164L2 | 9 | KLSQHMTEV | 122 |
| p53.216 | 10 | VVVPYEPPEV | 617 |
| p53.216L2 | 10 | VLVPYEPPEV | 89 |
| p53.229 | 9 | CTTIHNYM | 278 |
| p53.229L2V9 | 9 | CLTIHNYV | 263 |
| p53.229B1L2V9 | 9 | BLTIHNYV | 116 |
| p53.236 | 8 | YMCNSSCM | 4546 |
| p53.236L2M8 | 8 | YLCNSSCV | — |
| p53.236 | 11 | YMCNSSCMGGM | 667 |
| p53.236L2M11 | 11 | YLCNSSCMGGV | 22 |
| p53.255 | 11 | ITLEDSSGNLL | 1563 |
| p53.255L2V11 | 11 | ILEDSSGNLV | 33 |
| p53.256 | 10 | TLEDSSGNLL | 1667 |
| p53.256V10 | 10 | TLEDSSGNLV | 4167 |

— indicates binding affinity $\geq 10,000$ nM.

Table XII. Crossbinding data of 12 fixed p53 A*0201 peptides.

| Source | AA | Sequence | A*0201 nM | A*0202 nM | A*0203 nM | A*0206 nM | A*6802 nM | No. A2 Alleles Crossbound |
|---------------|----|--------------|--------------|--------------|--------------|--------------|--------------|---------------------------------|
| p53.69 | 8 | AAPVAPA | 5000 | 1536 | 1177 | 1233 | 4706 | 0 |
| p53.69L2V8 | 8 | ALPPVAPV | 217 | 7167 | 500 | 285 | 67 | 4 |
| p53.101 | 11 | KTYQGSYGRLL | 1786 | 896 | — | 514 | 615 | 0 |
| p53.101L2V11 | 11 | KLYQGSYGRRV | 81 | 48 | 24 | 116 | — | 4 |
| p53.129 | 9 | ALNKMFCQL | 735 | 391 | 19 | 73 | — | 3 |
| p53.129V9 | 9 | ALNKMFCQV | 75 | 165 | 7.7 | 15 | — | 4 |
| p53.129B7V9 | 9 | ALNKMFBQV | 192 | 391 | 23 | 49 | — | 4 |
| p53.129 | 10 | ALNKMFCQLA | 1316 | 1075 | 71 | 4625 | — | 1 |
| p53.129V10 | 10 | ALNKMFCQLV | 217 | 287 | 71 | 7400 | — | 3 |
| p53.132 | 9 | KMECQLAKT | 333 | 33 | 18 | 106 | — | 4 |
| p53.132V9 | 9 | KMECQLAKV | 33 | 8.4 | 7.7 | 15 | — | 4 |
| p53.132B4V9 | 9 | KMEBQLAKV | 125 | 13 | 9.1 | 37 | 8889 | 4 |
| p53.132L2V9 | 9 | KLFCQLAKV | 98 | 3.6 | 3.4 | 10 | 1270 | 4 |
| p53.135 | 9 | COLAKTGPV | 208 | 43 | 143 | 90 | — | 4 |
| p53.135L2 | 9 | CLAKTGPV | 125 | 506 | 67 | 370 | — | 3 |
| p53.135B1B7 | 9 | BOLAKTBPV | 102 | 71 | 15 | 67 | — | 4 |
| p53.135B1L2B7 | 9 | BLAKTBPV | 46 | 119 | 7.7 | 64 | — | 4 |
| p53.139 | 9 | KTCPVQLWV | 725 | 606 | 217 | 15 | — | 2 |
| p53.139L2 | 9 | KLCPVQLWV | 122 | 239 | 29 | 23 | — | 4 |
| p53.139L2B3 | 9 | KLBPVQLWV | 46 | 29 | 19 | 31 | — | 4 |
| p53.149 | 9 | STPPGTRV | 909 | 1162 | 1031 | — | 129 | 1 |
| p53.149M2 | 9 | SMPPGTRV | 172 | 215 | 13 | 425 | 667 | 4 |
| p53.149L2 | 9 | SLPPGTRV | 122 | 226 | 13 | 9250 | 140 | 4 |
| p53.164 | 9 | KOSQHMTFV | 500 | 130 | 170 | 285 | — | 4 |
| p53.164L2 | 9 | KLSQHMTFV | 122 | 94 | 35 | 46 | — | 4 |
| p53.216 | 10 | VVPYEPEPV | 617 | 1870 | 455 | 1194 | — | 1 |
| p53.216L2 | 10 | VLVPYEPEPV | 89 | 391 | 71 | 2056 | — | 3 |
| p53.236 | 11 | YMCNSSCMGGM | 667 | 391 | 67 | 974 | 5333 | 2 |
| p53.236L2M11 | 11 | YLCNSSCMGCV | 22 | 13 | 3.6 | 18 | 1569 | 4 |
| p53.255 | 11 | ITLEDSSGNLL | 1563 | 1265 | 2857 | 507 | 6667 | 0 |
| p53.255L2V11 | 11 | ITLEDSSGNLLV | 33 | 123 | 71 | 206 | — | 4 |

— indicates binding affinity $\geq 10,000$ nM.

Table XIII. Immunogenicity of 7 fixed p53 A*0201 peptides.

| Source | AA | Sequence | A*0201 nM | A*0202 nM | A*0203 nM | A*0206 nM | A*6802 nM | No. A2 Alleles Crossbound | CTL Peptide ¹ | CTL Wild-type | CTL Tumor |
|--------------|----|--------------|--------------|--------------|--------------|--------------|----------------|---------------------------------|-----------------------------|------------------|--------------|
| p53.69 | 8 | AAPVAPA | 5000 | 1536 | 1177 | 1233 | 4706 | 0 | | | |
| p53.69L2V8 | 8 | ALPPVAPV | 217 | 7167 | 500 | 285 | 67 | 4 | 2/4 | 1/3 | 0/3 |
| p53.129 | 9 | ALNKMFCQL | 735 | 391 | 19 | 73 | — ¹ | 3 | | | |
| p53.129V9 | 9 | ALNKMFCQV | 75 | 165 | 7.7 | 15 | — | 4 | 0/1 | | |
| p53.129B7V9 | 9 | ALNKMFBQV | 192 | 391 | 23 | 49 | — | 4 | 2/4 | 0/3 | 0/2 |
| p53.132 | 9 | KMFCQLAKT | 333 | 33 | 18 | 106 | — | 4 | | | |
| p53.132V9 | 9 | KMFCQLAKV | 33 | 8.4 | 7.7 | 15 | — | 4 | 1/3 | 0/2 | 0/2 |
| p53.132B4V9 | 9 | KMFBQLAKV | 125 | 13 | 9.1 | 37 | 8889 | 4 | 5/5 | 0/4 | 0/4 |
| p53.132L2V9 | 9 | KLFCQLAKV | 98 | 3.6 | 3.4 | 9.5 | 1270 | 4 | 2/3 | 1/3 | 0/3 |
| p53.139 | 9 | KTCPVQLWV | 725 | 606 | 217 | 15 | — | 2 | | | |
| p53.139L2 | 9 | KLCPVQLWV | 122 | 239 | 29 | 23 | — | 4 | 2/5 | 2/3 | 1/3 |
| p53.139L2B3 | 9 | KLBPVQLWV | 45 | 29 | 19 | 31 | — | 4 | 3/4 | 2/3 | 1/2 |
| p53.149 | 9 | STPPGCTRV | 909 | 1162 | 1031 | — | 129 | 1 | | | |
| p53.149L2 | 9 | SLPPGCTRV | 122 | 226 | 13 | 9250 | 140 | 4 | 2/3 | 1/3 | 0/3 |
| p53.149M2 | 9 | SMPPPGCTRV | 172 | 215 | 13 | 425 | 667 | 4 | 2/4 | 2/4 | 2/4 |
| p53.216 | 10 | VVVPVEPEPEV | 617 | 1870 | 455 | 1194 | — | 1 | | | |
| p53.216L2 | 10 | VLVPVEPEPEV | 89 | 391 | 71 | 2056 | — | 3 | 1/1 | 1/1 | |
| p53.255 | 11 | ITLEDSSGNLL | 1563 | 1265 | 2857 | 507 | 6667 | 0 | | | |
| p53.255L2V11 | 11 | ITLEDSSGNLLV | 33 | 123 | 71 | 206 | — | 4 | 1/3 | 0/3 | 0/2 |

1) Number of donors yielding a positive response/total tested.

2) — indicates binding affinity $\geq 10,000$ nM.

Table XIV. Crossbinding data of 5 fixed MAGE2/3 A*0201 peptides.

| Source | AA | Sequence | A*0201 nM | A*0202 nM | A*0203 nM | A*0206 nM | A*6802 nM | No. A2 Alleles Crossbound |
|---------------|----|------------|--------------|--------------|--------------|--------------|--------------|---------------------------------|
| MAGE3.112 | 9 | KVAELVHFL | 69 | 29 | 14 | 168 | 17 | 5 |
| MAGE3.112L2 | 9 | KLAEVLVHFL | 20 | 6.0 | 5.9 | 12 | 400 | 5 |
| MAGE3.112M2 | 9 | KMAELVHFL | 24 | 6.7 | 7.7 | 26 | 286 | 5 |
| MAGE3.112L2V9 | 9 | KLAEVLVHFV | 14 | 13 | 22 | 15 | 73 | 5 |
| MAGE3.112M2V9 | 9 | KMAELVHFV | 26 | 17 | 46 | 39 | 170 | 5 |
| MAGE3.220 | 9 | KIWEELSVL | 333 | 391 | 2381 | 308 | -- | 3 |
| MAGE3.220L2V9 | 9 | KLWEELSVV | 11 | 165 | 20 | 15 | -- | 4 |

-- indicates binding affinity $\geq 10,000$ nM.

Table XV. Crossbinding data of 6 fixed Her2/neu A*0201 peptides.

| Source | AA | Sequence | A*0201 nM | A*0202 nM | A*0203 nM | A*0206 nM | A*6802 nM | No. A2 Alleles Crossbound |
|---------------------|----|------------|--------------|--------------|--------------|--------------|--------------|---------------------------------|
| Her2/neu.5 | 9 | ALCRWGILL | 100 | -- | 278 | -- | -- | 2 |
| Her2/neu.5B3V9 | 9 | ALBRWGILLV | 18 | 33 | 4.2 | 285 | -- | 4 |
| Her2/neu.5M2B3V9 | 9 | AMBRWGILLV | 36 | 473 | 16 | 726 | -- | 3 |
| Her2/neu.153 | 9 | VLIQRNPQL | 23 | 3909 | 3.3 | 1057 | -- | 2 |
| Her2/neu.153V9 | 9 | VLIQRNPQV | 55 | 768 | 135 | 385 | -- | 3 |
| Her2/neu.369 | 9 | KIFGSLAFL | 36 | 9.0 | 19 | 23 | 3333 | 4 |
| Her2/neu.369L2V9 | 9 | KLFGSLAFV | 5.8 | 7.5 | 19 | 17 | 1270 | 4 |
| Her2/neu.653 | 9 | SISAVVGI | 69 | 524 | 35 | 285 | 148 | 4 |
| Her2/neu.653.L2V9 | 9 | SISAVVGV | 7.1 | 10 | 16 | 20 | 110 | 5 |
| Her2/neu.665 | 9 | VVLGVVFGI | 14 | -- | 2500 | 430 | 2000 | 2 |
| Her2/neu.665L2V9 | 9 | VVLGVVFGV | 2.4 | 19 | 14 | 6.0 | 8000 | 4 |
| Her2/neu.952 | 10 | YMMVVKCWM | 20 | 307 | 83 | 116 | 267 | 5 |
| Her2/neu.952L2V10 | 10 | YLMVVKCWMV | 13 | 56 | 116 | 18 | 84 | 5 |
| Her2/neu.952L2B7V10 | 10 | YLMVVKBWMV | 7.2 | 66 | 77 | 11 | 851 | 4 |

-- indicates binding affinity $\geq 10,000$ nM.

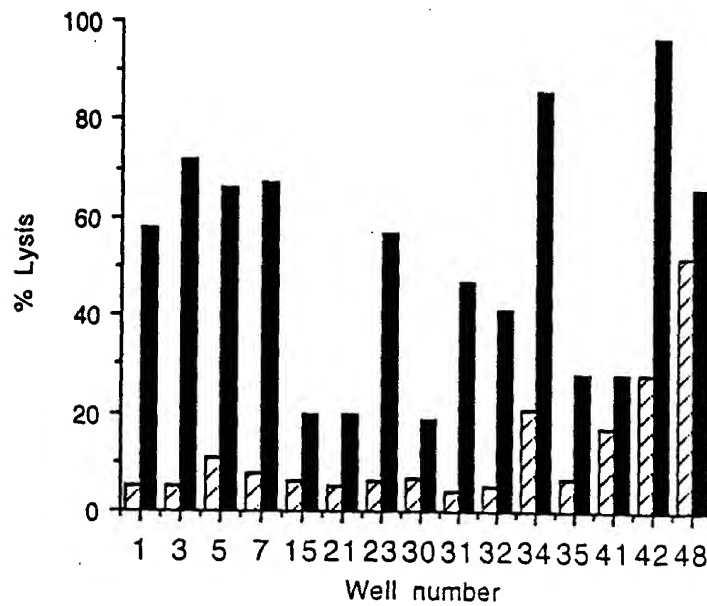
Table XVI. Immunogenicity of fixed Her2/neu peptides.

| Source | Sequence | A*0201 nM | A*0202 nM | A*0203 nM | A*0206 nM | A*6802 nM | No. A2 Alleles Crossbound | CTL Peptide ¹ | CTL Wild-type ¹ | CTL Tumor ¹ |
|---------------------|--------------|--------------|----------------|--------------|--------------|--------------|---------------------------------|-----------------------------|-------------------------------|---------------------------|
| Her2/neu.5 | ALCRWGILL | 100 | — ² | 278 | — | — | 2 | | 2/2 | 2/2 |
| Her2/neu.5B3V9 | ALBRWGILLV | 18 | 33 | 4.2 | 285 | — | 4 | 2/3 | nt | 0/3 |
| Her2/neu.5M2V9 | AMCRWGILLV | 179 | 7167 | 63 | 128 | — | 3 | 1/2 | nt | 0/2 |
| Her2/neu.952 | YMDIMVKCWMII | 20 | 307 | 83 | 116 | 267 | 5 | | 0/1 | 0/1 |
| Her2/neu.952L2B7V10 | YLIIMVKBWMIV | 7.2 | 66 | 77 | 11 | 851 | 4 | 3/3 | nt | 0/3 |

1) Number of donors yielding a positive response/total tested.

2) — indicates binding affinity $\geq 10,000$ nM.

Figure 1.



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Figure 2a.

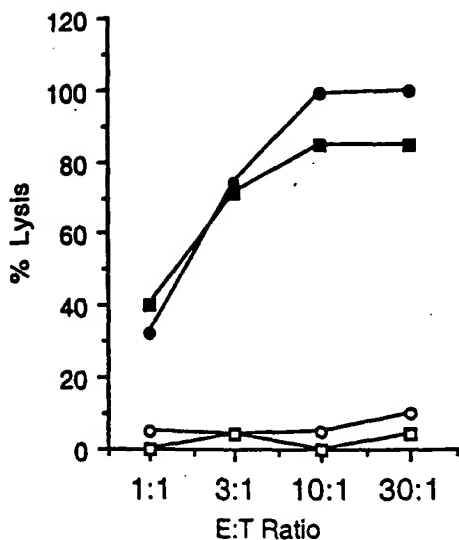


Figure 2b.

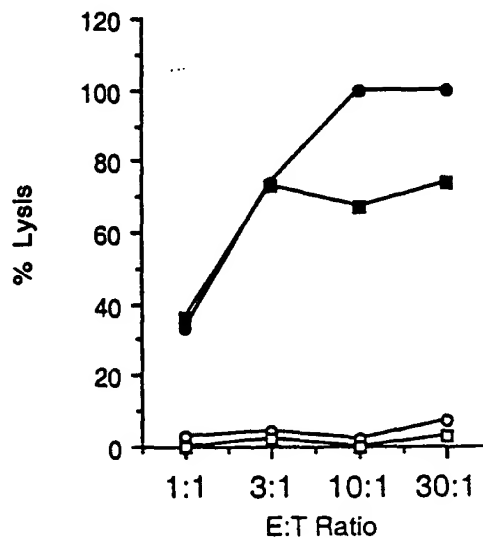


Figure 2c.

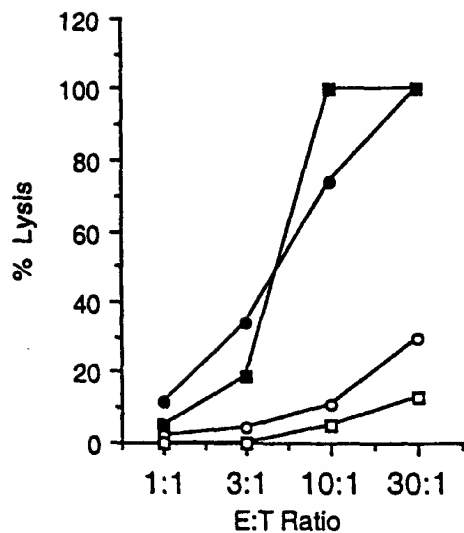
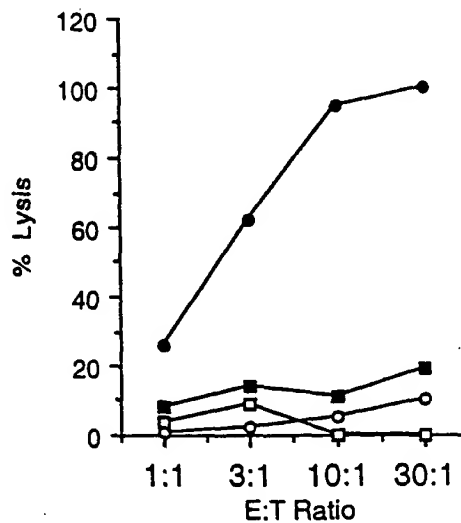


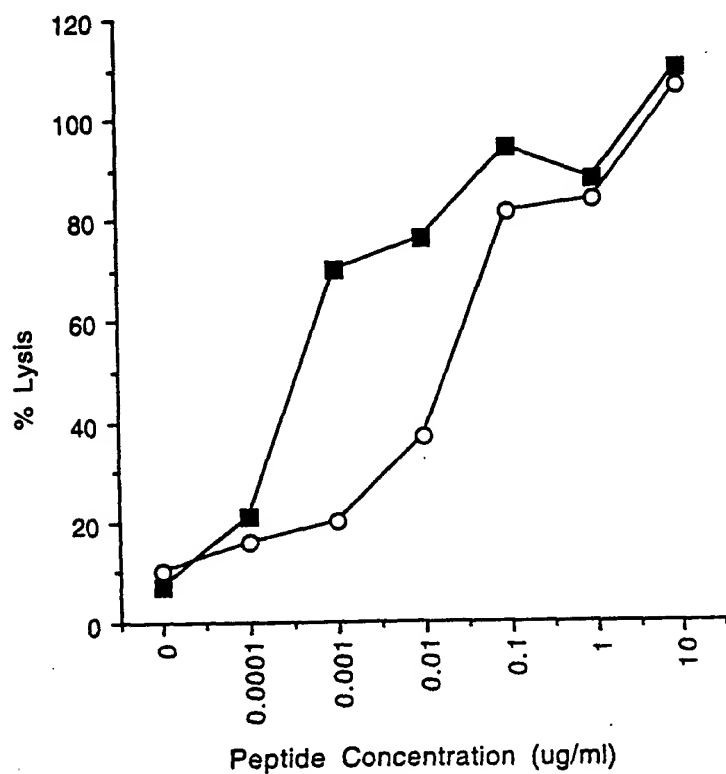
Figure 2d.



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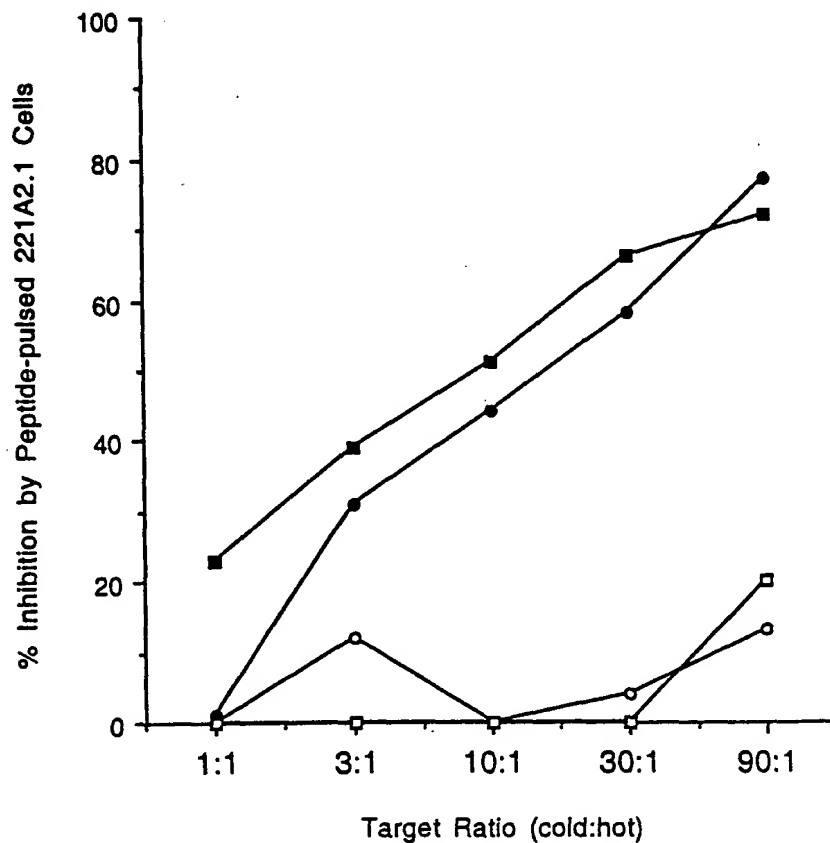
Figure 3.



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Figure 4.



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Figure 5b.

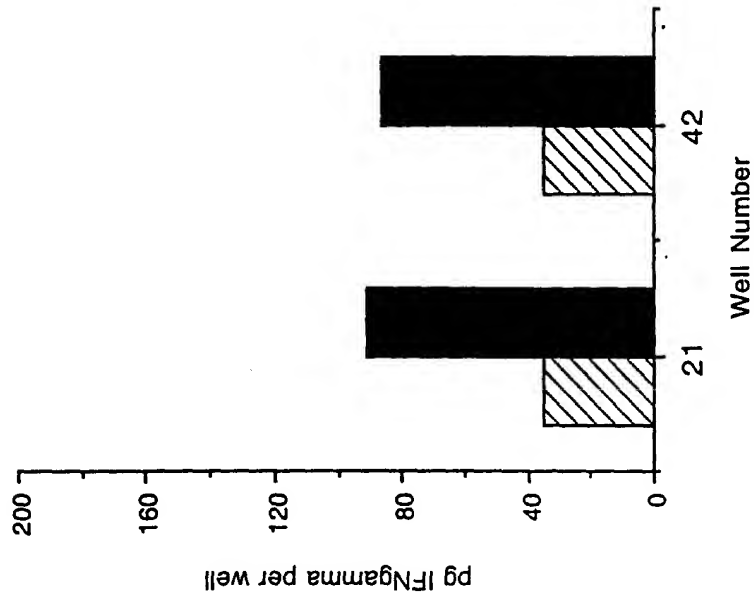
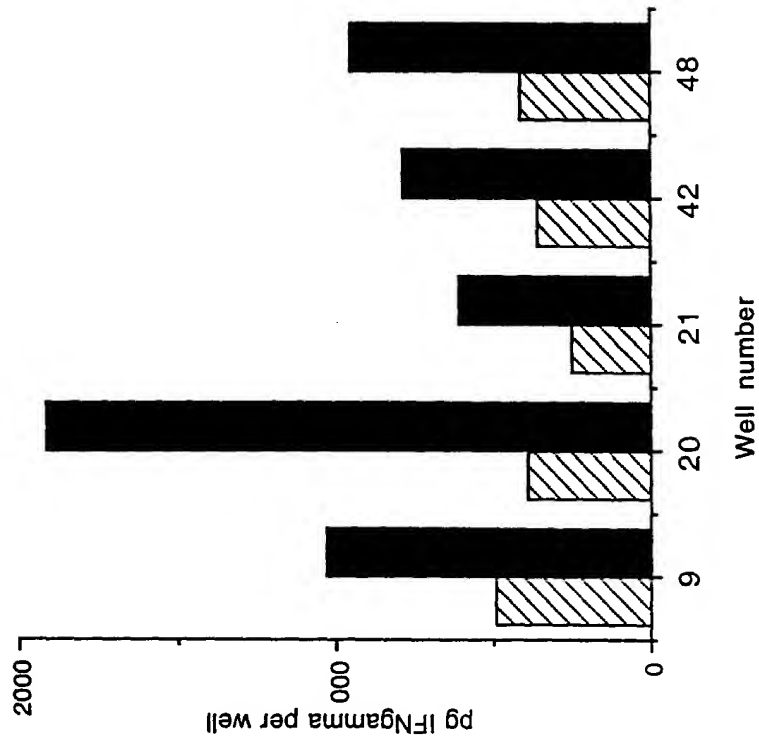


Figure 5a.



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Figure 6b.

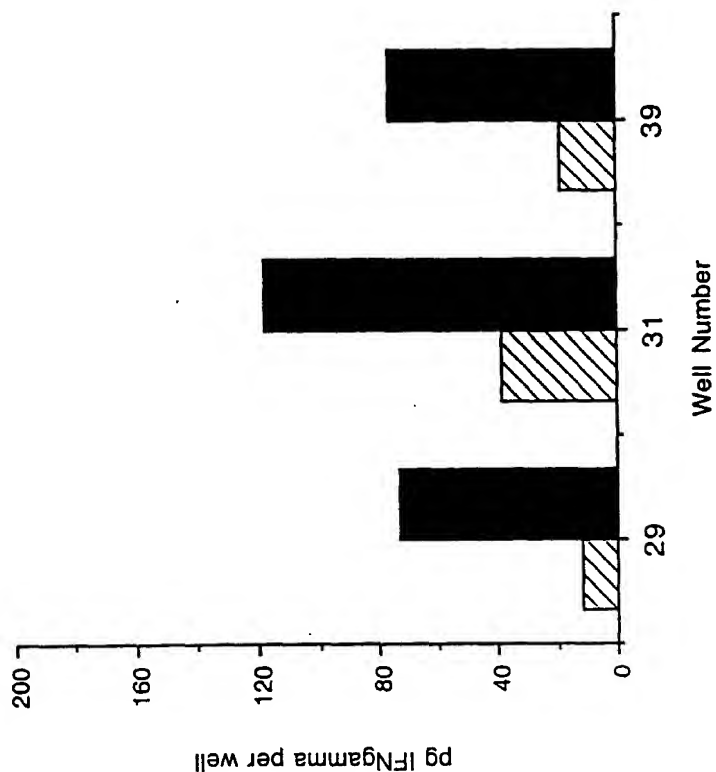
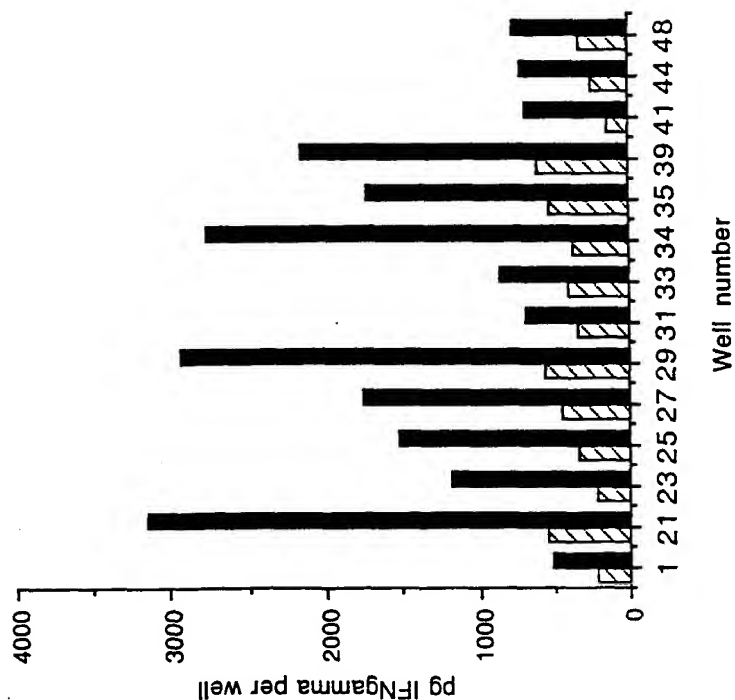


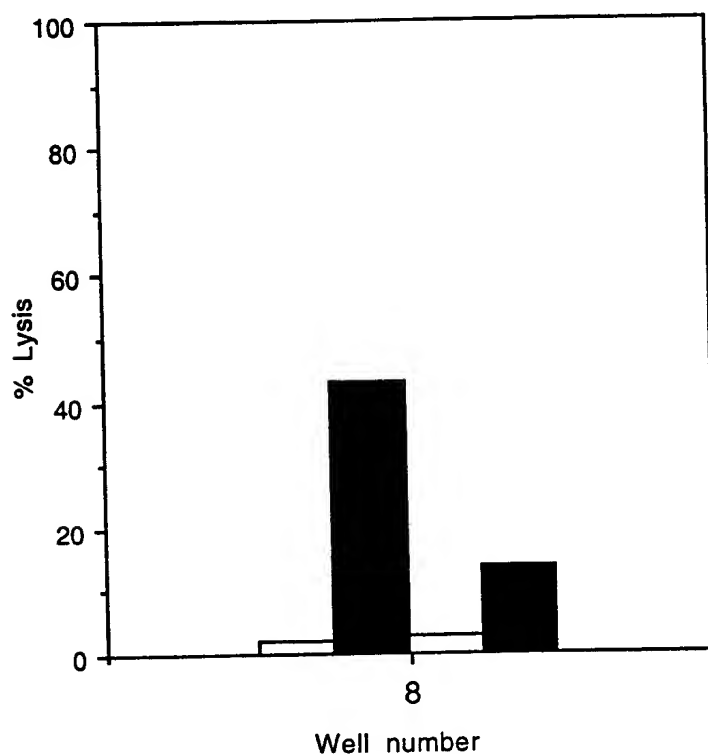
Figure 6a.



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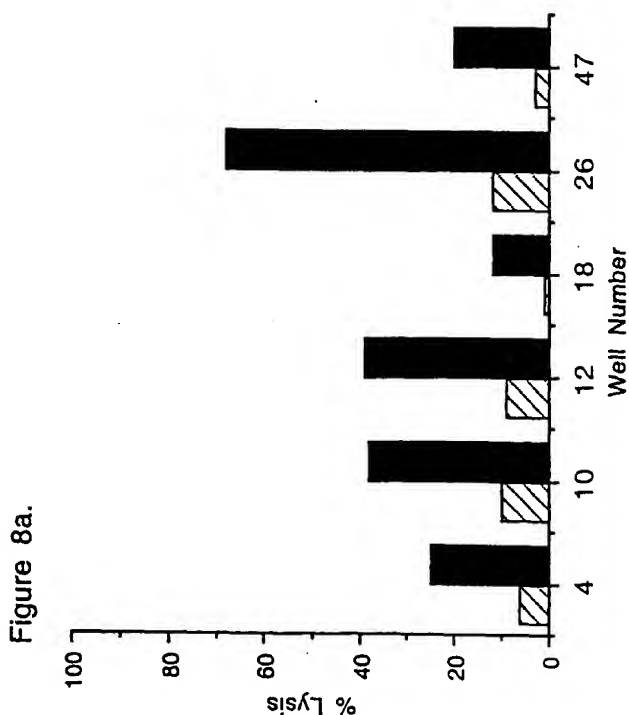
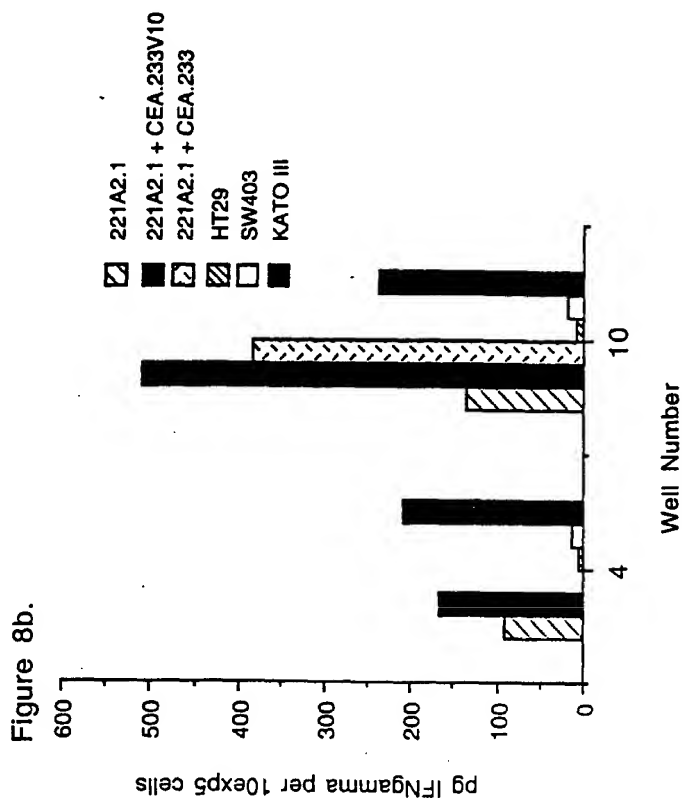
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Figure 7.



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Figure 9b.

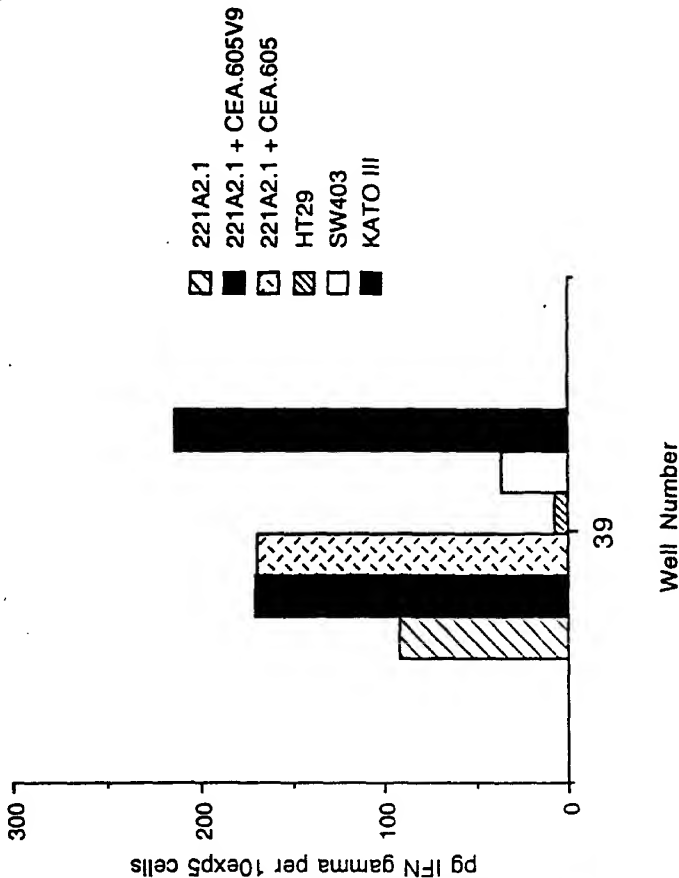
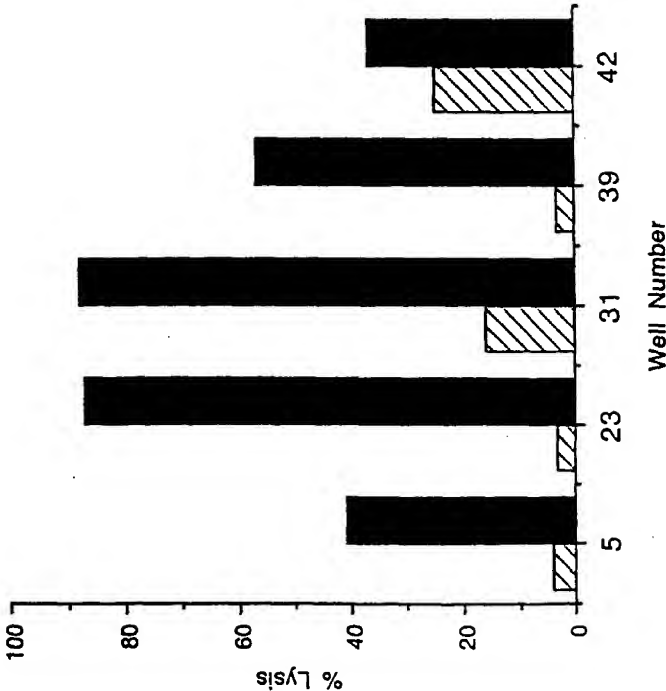
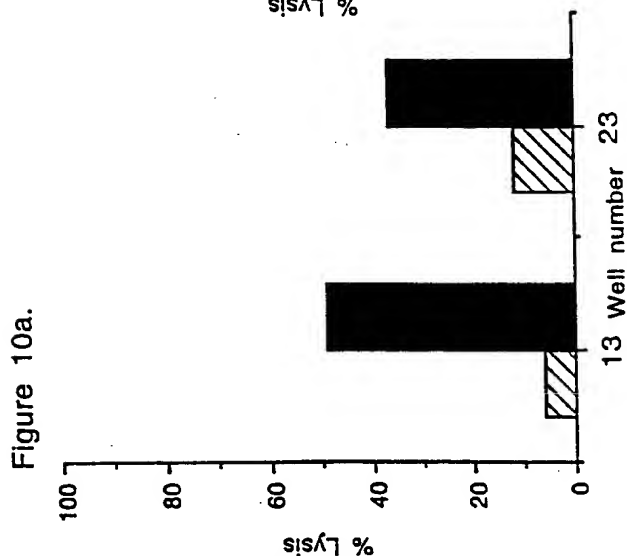
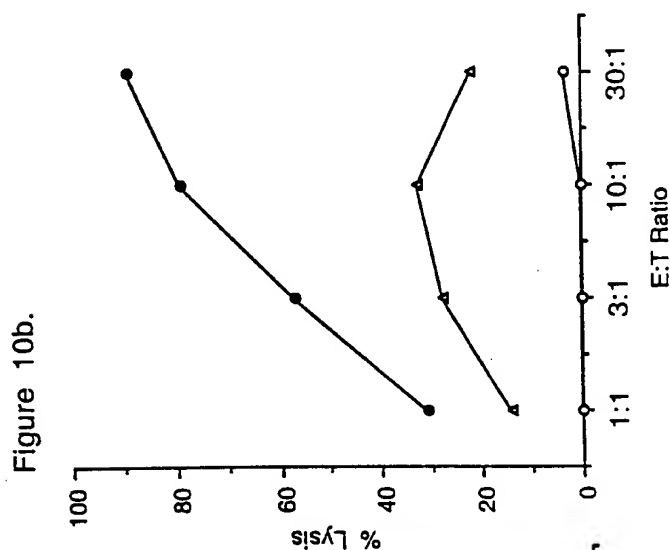
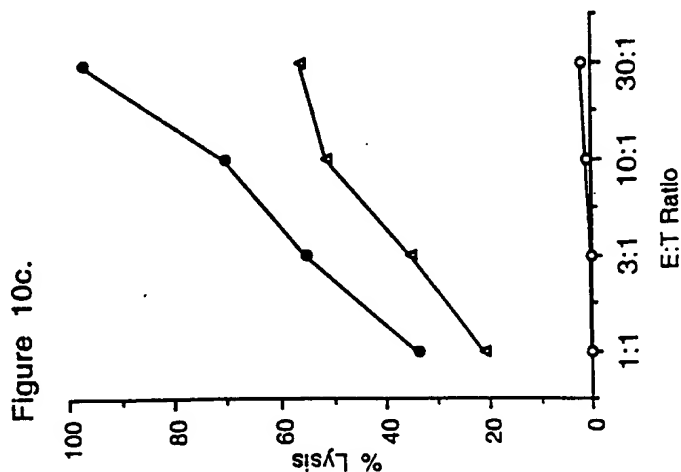


Figure 9a.



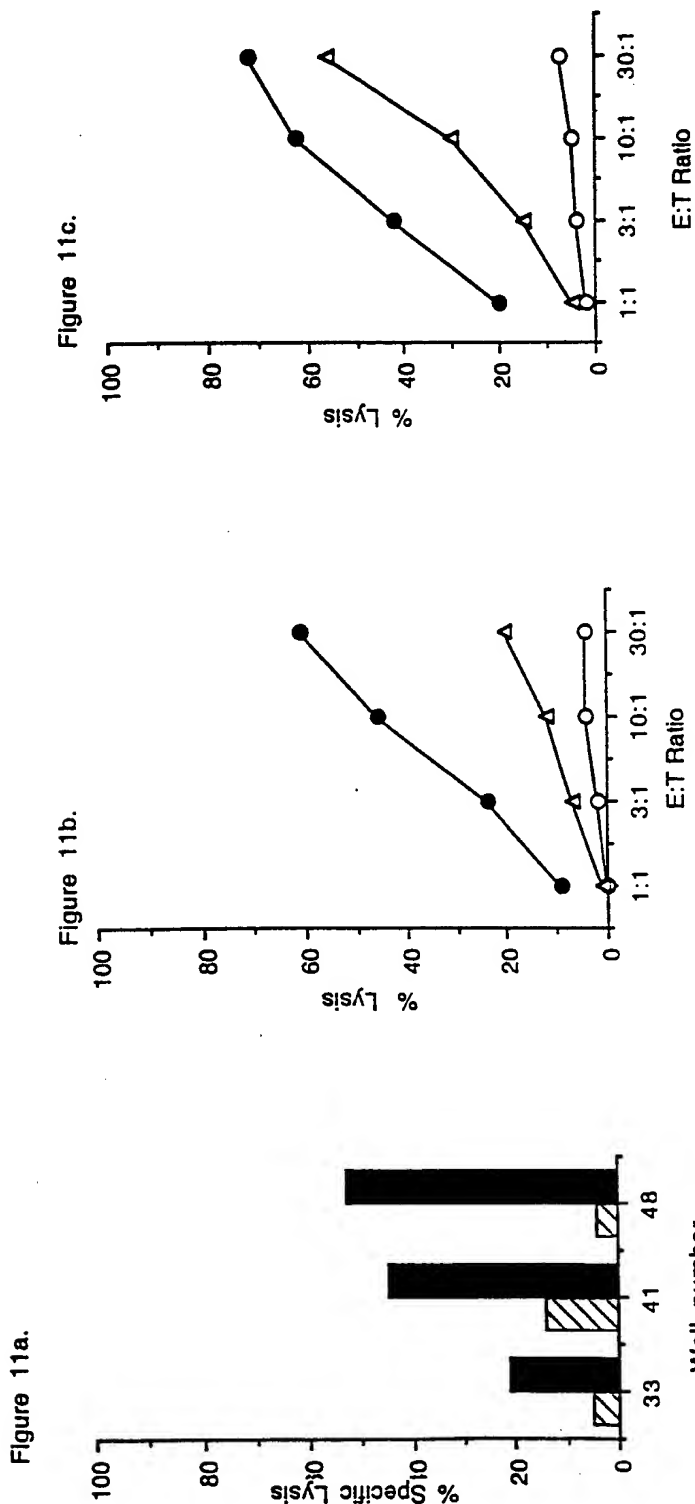
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Figure 12a.

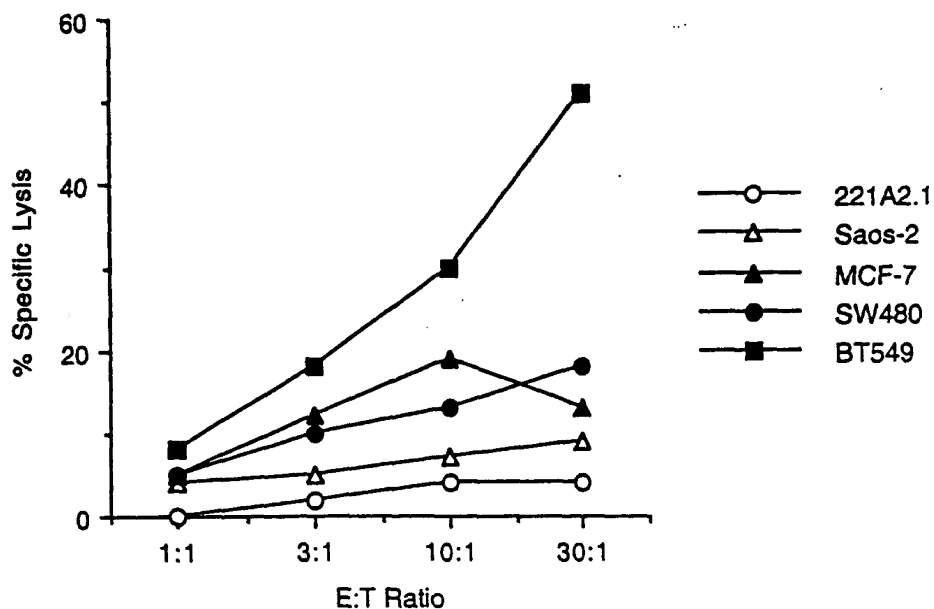
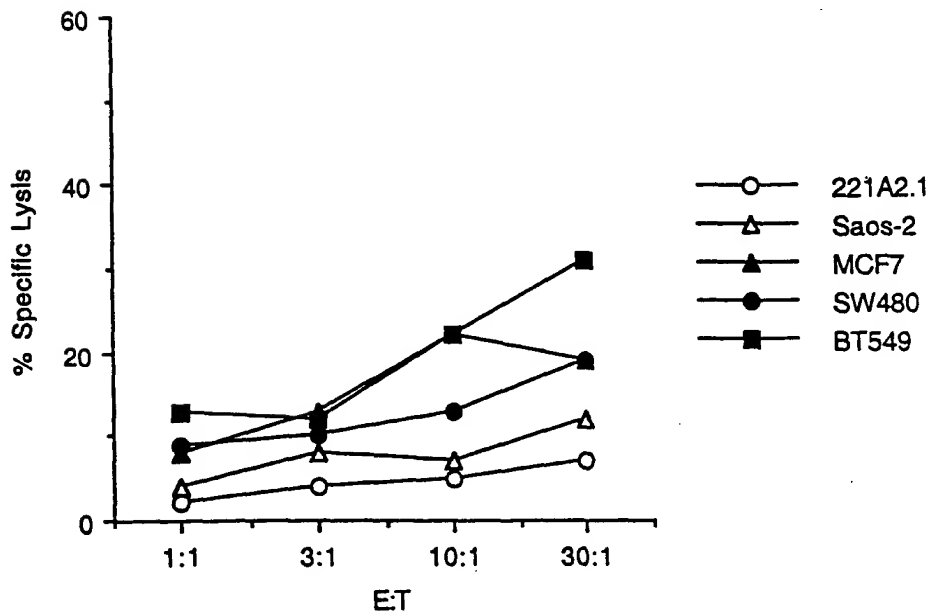


Figure 12b.



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Figure 13a.

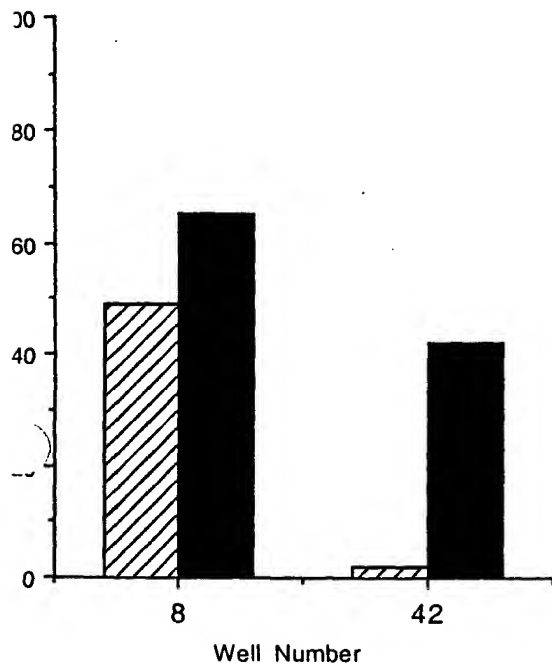
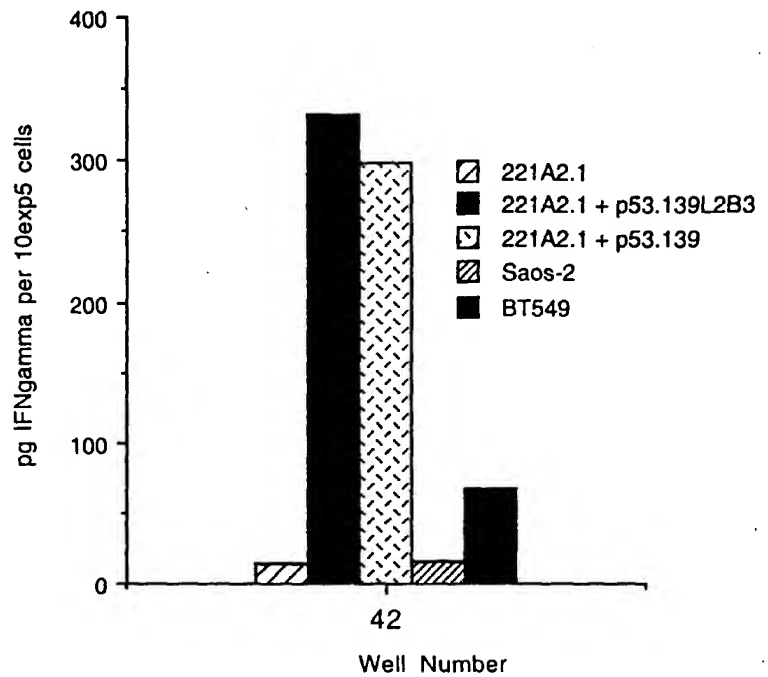


Figure 13b.



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Figure 14a.

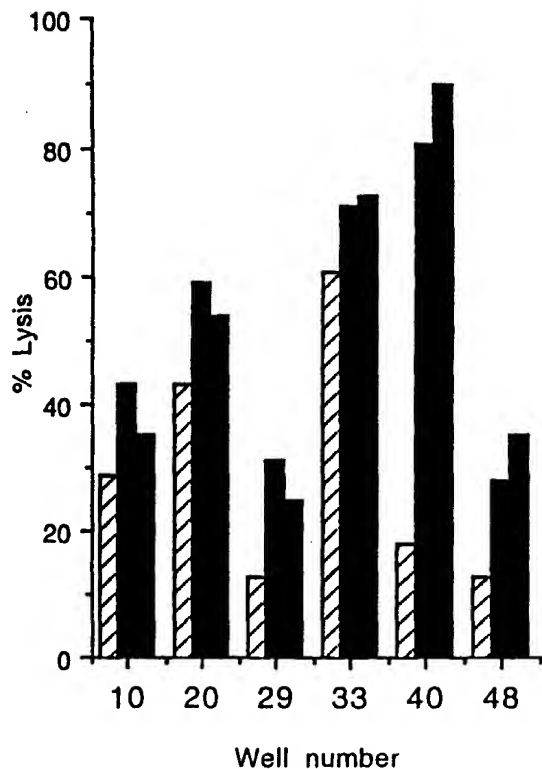
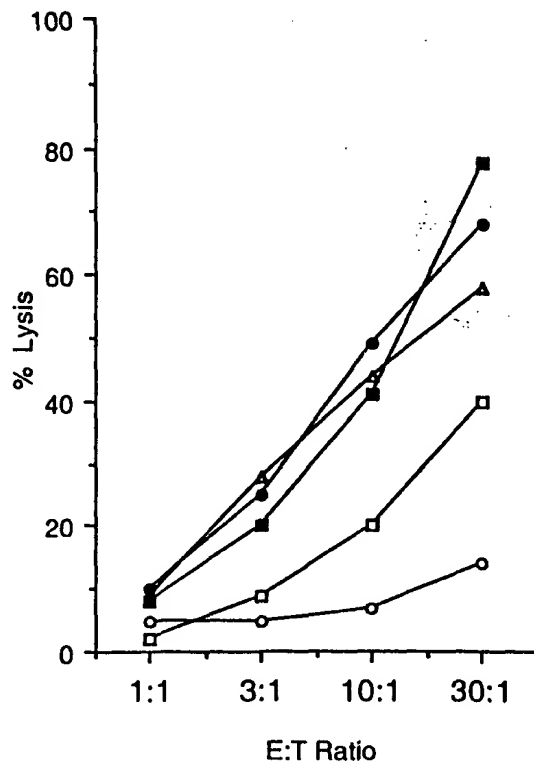


Figure 14b.



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